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IN SITU CULTURES OF DERMATOPHYTES[1]

BY A. M. DAVIDSON[2] AND P. H. GREGORY[3]

Abstract

Studies were undertaken to correlate structure with life cycle in four common species of fungi causing skin disease in man. Hairs naturally infected by *Microsporon audouini*, *M. felineum*, and *Trichophyton gypseum* and scutula of *Achorion schoenleini* were placed, without any nutrient medium, in van Tieghem cells at various humidities controlled by osmotic solutions of known vapor pressure. These fungi, which while parasitizing the animal body produce hyphae and thallospores only, were then found to undergo a second period of growth on the detached infected host tissue in moist atmospheres. During this phase they produced all the highly differentiated spore forms previously known only on various media. The aerial hyphae, aleuriospores, fuseaux and spirals produced by such *in situ* cultures are described in some detail. It is suggested that in nature the saprophytic phase is initiated when the infected tissues fall from the animal body in a moist situation, and that this phase may have significance in the epidemiology of the disease.

Introduction

At least 180 species of dermatophytes have been mentioned in the literature as pathogenic to man. However, there is not a single species whose biology has been adequately investigated; in particular the significance of many of the structures found in the life cycle of these fungi is entirely unknown. The studies described in this paper were undertaken in an attempt to correlate structure with life cycle in the dermatophytes, and the paper forms an extended account of work which has already received preliminary notice in Nature (3).

The dermatophytes, in the narrow sense in which the word is used in the present paper, comprise those skin inhabiting fungi which cause favus and ringworm in its various manifestations. The dermatophytes form a physiological group of fungi capable of attacking such keratinized structures as the stratum corneum, hair, nails, horns, hooves and feathers. It is now generally believed on morphological grounds that, in addition to forming a physiological group, they are related taxonomically. The evidence presented in this paper tends to support this view.

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The ringworm fungus while infecting the hair consists of: (i) hyphae running longitudinally within the hair sheath or the shaft of the hair; and (ii) small round chlamydospores formed by the rounding-off and disarticulation of the hyphae, and therefore to be regarded as oidia or thallospores. The structure of the principal dermatophytes in their pathogenic phase has been dealt with amply by Sabouraud (8). It is a phase of limited structure, characterized by the production of immense numbers of thallospores, or in favus of compact masses of hyphae known as scutula.

On the other hand when growing on artificial media most dermatophytes produce conidia. Instead of the thallospores, differing little from one species to another, which characterize the dermatophytes in the hair, we find a variety of more highly differentiated structures which are often characteristic for different species. The morphology of the dermatophytes in culture has also been dealt with by Sabouraud (8) and various subsequent writers. Among the structures produced in culture may be mentioned: (i) the thick walled fuseaux or macroconidia of *Microsporon felineum*, which in shape somewhat resemble the conidia of a Helminthosporium; (ii) the aleuriospores or conidia produced on branched conidiophores by the majority of the dermatophytes; and (iii) the helically twisted hyphae or spirals found in cultures of members of the Trichophyton gypseum group. These conspicuous organs are well formed and often abundant in cultures, yet they are not observed while the fungus is living on the animal body. It is difficult to believe that such highly differentiated structures as those enumerated above are artifacts due to the unusual conditions of the culture tube, or that structures so regular in form and abundantly produced are merely atavisms. Most investigators have recognized that these organs point to the existence of a saprophytic phase in the life history of the dermatophytes yet undiscovered in nature.

Brocq-Rousseu, Urbain and Barotte (1) grew *Trichophyton gypseum* on the sterilized litter and fodder of stable animals, and reported that in the saprophytic condition the vitality and virulence of cultures on straw were retained for over two years. Langeron and Milochevitch (6) investigated the microscopic structure on such media and discovered that natural substances such as straw and grains of cereals are exceptionally favorable to the production of the more complex dermatophyte organs. They accordingly expressed the opinion that the life cycle of these fungi includes a phase on materials relatively poor in nutriment. The writers have adopted a somewhat different hypothesis, but one which, however, by no means excludes the view of the saprophytic phase expressed by Langeron and Milochevitch.

The evidence from cultures for the existence of spore forms and other structures in the dermatophytes which have never been found in nature formed the starting point of the enquiry.

The most obvious clinical feature of ringworm, both in man and animals, is the penetration of hair and epidermis by the fungus, followed by the falling of infected hairs and scales from the body. Ringworm is thus characterized by defluvium in hairy regions or desquamation of the glabrous skin. In

animals, less frequently in civilized man, this detached infected material must often fall into moist places such as among vegetation or humus. Arrived there it might either colonize the substratum upon which it finds itself, as assumed by the Parisian school, or on the other hand might continue growth under the new conditions by the absorption of water by the fungus present in the detached hair, scale or scutulum, without the necessity of colonizing new material.

As far back as 1902 Plaut (7) placed trichophyton-infected hairs in a moist environment and observed the development of mycelium and "ectospores". This important experiment appears to have been lost sight of by subsequent workers, and its significance in the life history of the ringworm organisms has never been appreciated. This work of Plaut was unfortunately not known to the writers when the preliminary communication was made of their investigation along the same lines, or reference would have been made to these interesting experiments which were carried out over 30 years ago.

Methods and Material

Experiments were designed to test the hypothesis that upon naturally infected hairs, scales or scutula detached from the lesions on the body the fungus could continue growth and produce spores. A few tests were sufficient to demonstrate the correctness of this hypothesis, and the following technique was developed during the course of several experiments. The object of the technique is to supply the fungus present in the infected material solely with water by exposing it to a moist atmosphere, and to withold all nutriment except that present in the infected tissue. Further, it was desired to expose the fungus to a range of humidities. This was achieved by placing the infected material in water vapor at various degrees of saturation.

The apparatus used was a slight modification of the van Tieghem hanging-drop cell. Each cell was set up in the following way. A clean flamed glass microscope slide 3 by 1 in. was laid flat on the table. The two cut ends of a sterilized glass ring, 18 mm. diameter by 10 mm. high, were dipped into melted white vaseline and quickly withdrawn, and the edges of the ring thus became coated with vaseline. The ring was then lowered on to the middle of the slide before the latter had completely cooled. The heat of the slide melted the vaseline in contact with it on the ring, and on further cooling a water tight union was formed between the slide and the ring. About 1 cc. of the solution determined upon to keep the atmosphere moist was then placed in the bottom of the cell by means of a pipette. Cover slips were carefully cleaned before use by boiling in alkali, rinsing in running tap water and wiping on a clean cloth. They were stored in alcohol until required. This was done to ensure that no soluble substances remained on the glass from which the fungus could derive nutriment or which might inhibit germination. Thus all growth was derived from food in the hair or scale. The cell was closed by lowering a clean, flamed cover slip on to the top of the ring and pressing it down into the vaseline deposited there.

The cell thus prepared was set aside for a few minutes to cool before being inoculated. As the cell cooled a film of water usually condensed like dew on the underside of the cover slip. This was used to attach the infected material to the cover slip. The cover slip was lifted off from the ring and the infected material quickly deposited in the water-film with sterile forceps or needle, and the cover slip replaced at once before the film had time to dry. Except in cells containing a saturated atmosphere the film usually evaporated again after a short time when equilibrium was reached in the cell, leaving the material reasonably securely attached to the cover of the cell. In the driest atmospheres employed in the experiments an adequate film sometimes failed to form. In these cells a small drop of sterile water was placed on the cover slip and the material placed in this before closing the cell. When the cell was closed the drop rapidly evaporated as before.

It was desired to place the hairs and other material under different humidities. The fact that the presence of a dissolved substance lowers the vapor tension of a liquid was utilized to supply various humidities to which the infected material was submitted. Sucrose was chosen as a solute with which to lower the vapor tension of water, as being a non-volatile substance easily weighed out, and highly soluble in water. Its non-volatility eliminated danger of sucrose molecules escaping from the liquid and serving as nutriment to the fungus in the infected tissue.

To prevent the growth of micro-organisms the humidifying solution also contained 1% of hydrated copper sulphate. This also served to remove the possibility that the fungus which grew out of the infected tissue might utilize the osmotic solution as a source of food by means of the long pendant aerial hyphae which are sometimes produced. Sucrose had the advantage over dextrose of not precipitating the copper sulphate added to the solution as a preservative.

The concentrations of sucrose which were principally employed to lower the vapor pressure over the solution were: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 molar. The approximate osmotic pressures and corresponding percentage humidities of the atmosphere in contact with solutions of various concentrations is shown in Table III. Throughout the paper the approximate humidities in the cells are expressed in percentage, and in each case the molarity of the sucrose solution used to produce the given humidity is shown in brackets.

The cells were left at the temperature of the laboratory, which remained within a few degrees of 23° C. during the period in which most of the experiments were conducted. Sudden falls in temperature of the room in which the cultures are kept are to be avoided as the atmosphere in the cells may become temporarily saturated before equilibrium is restored. Under these conditions water is deposited on the surface of the cultures, often destroying the natural arrangement of the organs.

For daily examination of the preparations a high power dry objective with a long working distance has been found useful, as deeper parts of the culture

can then be safely examined without danger of the objective touching the cover slip. To prevent heating of the cultures a heat absorbent was placed between the source of light and the condenser of the microscope. In the lower humidities, at least, the hyphae were in air, consequently it was impossible to make out details of the structure, such as septa, which were often invisible until the mycelium was mounted in some refractile medium.

The following technique has been found useful in rapidly making permanent preparations. The cover slip was removed from the cell, inverted, and a drop or two of glacial acetic acid run over the culture to fix it. While in the acetic acid most of the vaseline was wiped off the cover slip with a cloth. After five minutes the cover slip was placed with the culture downwards on the surface of a vessel containing distilled water. After several washings in distilled water over a period of three to four hours the culture while still wet was lowered on to a drop of melted glycerine jelly containing methylene blue on a slide.

In the preparation of the cells for the experiment sterile precautions were taken throughout. The hairs, scales and scutula, on the other hand, were placed in the cells just as they were taken from the body, without any attempt at sterilizing the outside of the material, although they were handled with sterile instruments in order to avoid contamination after being removed from the body. As would be expected under these conditions contamination of the cultures by saprophytic organisms was found occasionally. Bacteria appeared only when the atmosphere in the cell was moist enough to deposit water around the infected material, that is, in a saturated or nearly saturated atmosphere. Their occurrence is dealt with below. Saprophytic molds were found to develop at any of the humidities tested. Their occurrence was however exceptional, and the mold principally encountered was, as would be expected, *Penicillium*. Although contaminants occurred in a small percentage of cultures they were not frequent. Their occurrence however calls for a knowledge of the structures of the dermatophytes in pure culture before conclusions are drawn from this technique.

The technique is easy to carry out and is eminently suited for class demonstration. Students could make their own preparations and study the structure of dermatophytes direct from clinical material without the necessity of maintaining cultures on Sabouraud's medium. For class or routine laboratory purposes a molar sugar solution with 1% copper sulphate would probably be most generally useful.

The writers have borrowed Plaut's term "*in situ* cultures" for preparations of the dermatophytes obtained by this new technique. According to the nature of the inoculum *in situ* cultures comprise: infected-hair cultures, infected-scale cultures, and scutulum cultures.

Naturally infected tissue, consisting of hairs, scales or scutula, was taken from patients and stored in the laboratory between sterilized glass slides in small envelopes. The fungus present in each specimen was first determined by means of cultures on proof agar, prepared with the maltose *brute de Chantt*

and peptone *granulée de Chassaing* as specified by Sabouraud. Other portions of the material, which thus contained fungi already identified, were used to inoculate the moist cells.

A large number of *in situ* cultures were prepared with: (i) hairs from seven children with scalp ringworm due to *Microsporon audouini;* (ii) hairs from three children with scalp ringworm, and from two kittens with ringworm, due to *Microsporon felineum;* (iii) hairs from two children with kerion celsi due to *Trichophyton gypseum;* (iv) hairs and scutula from three children with favus capitis due to *Achorion schoenleini.*

The four common dermatophyte species studied have been described in the parasitic phase and in culture on various media by previous writers. The following descriptions of *in situ* cultures are intended to supplement the published descriptions of these fungi with an account of their morphology in the saprophytic phase on the host tissue. The descriptions are from notes and drawings made while the cultures were growing, and also from a study of preparations mounted in glycerine jelly. All the photographs are from living preparations in air.

Microsporon audouini

Microsporon audouini Gruby. *In situ* cultures: *aerial hyphae*, somewhat sinuous, diameter 1.5–3 μ; *aleuriophores* (conidiophores), long, erect, usually unbranched; *aleuriospores* (conidia), hyaline, unicellular, borne singly on lateral wall of hypha, sessile or on short necks, pyriform to oblong, proximal end faceted, distal end round, about 3–5 $\times$ 1.5–2 μ; *fuseaux* (macroconidia), sparse, hyaline, smooth, wall somewhat thickened, cylindrical to clavate, proximal end faceted, distal end round, 2- to 4-septate, 30–70 $\times$ 6–10 μ; *spirals* absent.

As shown in Table I the time required for the fungus in the infected hair to germinate was found to depend upon the humidity of the atmosphere in the moist cell. The germ tubes which emerged from the spore sheath around the hair were narrow sinuous hyphae when produced in a saturated atmosphere, but appeared straighter under drier conditions.

TABLE I

RELATION BETWEEN HUMIDITY AND GERMINATION

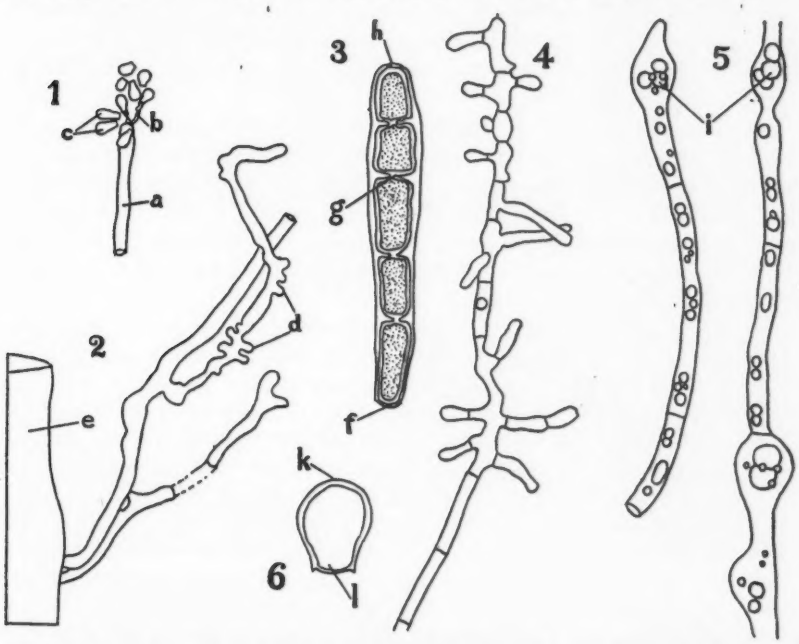
Conc. of humidifying soln., moles per litre	0.0	0.5	1.0	1.5	2.0
Germination time, days	1	2	5	10 Very poor	No germination

Aerial mycelium, which was well developed in cells of 98% humidity (1.0 *M*)*, consisted of straight or sometimes slightly sinuous hyphae of uniform diameter 1.5–3.0 μ wide, septate at intervals of 20 to 80 μ. The

*The figures in brackets show the molarity (*M*) of the sucrose solution used to produce the humidity indicated.

aerial hyphae were sparingly branched, usually midway between two septa and at right angles to the parent hypha. The tips of the young aerial hyphae were often seen bearing conspicuous drops of liquid. Numerous hyphal fusions commonly occurred in the aerial mycelium after a few days.

In the saturated atmosphere over the 0.0 *M* humidifying solution, no conidia were observed and the mycelium, which remained in the water-film which accumulated around the hair, appeared abnormal. Branching was irregular and the hyphae were contorted and had irregular denticulate



FIGS. 1–6. *Microsporon audouini. Infected-hair cultures.* FIG. 1. *Group of aleuriospores:* a, *living portion of aleuriophore;* b, *part of aleuriophore whose collapse has massed together the aleuriospores* c. FIG. 2. *Pectinate hyphae,* d; *arising from portion of infected hair,* e. FIG. 3. *Fuseau in glycerine jelly, shown in optical section;* f, *faceted end;* g, *pore in septum;* h, *rounded apex.* FIG. 4. *Aleuriophore with short branches.* FIG. 5. *Senile hyphae containing oil drops, i.* FIG. 6. *Diagram of aleuriospore:* k, *rounded apex;* l, *faceted proximal end. Magnifications: No. 1,* × *485; Nos. 2–5,* × *1100. Humidities: Nos. 1 and 4, 99% (0.5 M); Nos. 2 and 5, 100% (0.0 M); No. 3, 98% (1.0 M).*

projections giving them the appearance, as shown in Fig. 2, of Sabouraud's pectinate organs. Portions of the hyphae behind the tips sometimes became evacuated. As this water-submerged mycelium aged, certain of the cells enlarged to form chlamydospores; usually however the wall remained unthickened, and finally after about eight weeks both the unaltered mycelial cells and the chlamydospores lost their granular protoplasmic appearance and came to contain merely a few spherical refractile bodies. Chlamydospores in this stage sometimes had a superficial resemblance to asci containing as-

cospores. Perhaps these structures are identical with the "askusähnlichen organen" described by Kambayashi (5) in aged liquid cultures of *Microsporon japonicum*. That the globular structures formed in the old infected-tissue cultures of *M. audouini* were really intracellular fat droplets and not ascospores is evidenced by the following properties: (i) the number of spherical bodies varied from one to many in a cell; (ii) their diameter varied from 0.6 μ to 4.3 μ; (iii) when examined with the oil immersion lens the bodies appeared homogeneous and showed no signs of division into cell wall and cytoplasm; (iv) the bodies did not stain with protoplasmic stains such as methylene blue but took up fat stains such as scarlet red avidly; and (v) so far from being confined to special enlarged organs they occurred in any cell of the aging mycelium (Fig. 5).

Aleuriospores (conidia), were borne on long, straight, aerial hyphae usually unbranched but sometimes showing one or two short branches at right angles as shown in Fig. 4. The aleuriophores were undifferentiated from the ordinary hyphae except that in the sporogenous portion of the hypha the septa were closer together. The hypha, emptied by the formation of the spores, tended to collapse thus bringing the spores together in an irregular mass as shown in Fig. 1.

The aleuriospores (conidia) were first noticed about seven days after germination of the fungus in the hair (Plate 1, Fig. 1). They differ from the spores produced in the hair while on the body in that they are borne singly on the lateral walls of the conidiophores and are sessile or on short necks at right angles to the aleuriophore. They are aleuriospores in the sense of Vuillemin, that is to say they are not constricted off from the parent hypha, but are cut off from the parent by a transverse septum at the base of the spore. They are liberated on mechanical contact or on the disintegration of the dead and emptied aleuriophore. They thus come to have a characteristic shape; the distal end is rounded as it is the apex of a hypha, but the proximal end is truncated or faceted, the facet representing the septum at the base of the spore (Fig. 6.). Aleuriospores (conidia) and fuseaux (macroconidia) when present, in all four species of dermatophyte studied were of this shape.

Fuseaux (macroconidia) were noted in a few cultures a day or two later than the aleuriospores but their occurrence in this species was infrequent. They were formed on aerial hyphae in the same position as the ordinary branches, that is, laterally near the middle of a vegetative cell which they left at right angles. The wall was thickened up to 1 μ, the thickening being greatest at the distal end of the fuseau (Fig. 3). The spore was divided by transverse septa into from three to five cells. The septa were perforated by a central pore as was seen by careful focusing on the septa when mounted in glycerine jelly. The mode of germination of the fuseaux was not observed. Both fuseaux and aleuriospores were produced at humidities slightly below saturation, namely, 99.5–100% (0.3–1.0 *M*). At 100% humidity (0.0 *M*) the mycelium appeared degenerate.

When infected-tissue cultures were transferred to proof medium the gross appearance of the resulting mycelia was typical of *Microsporon audouini*.

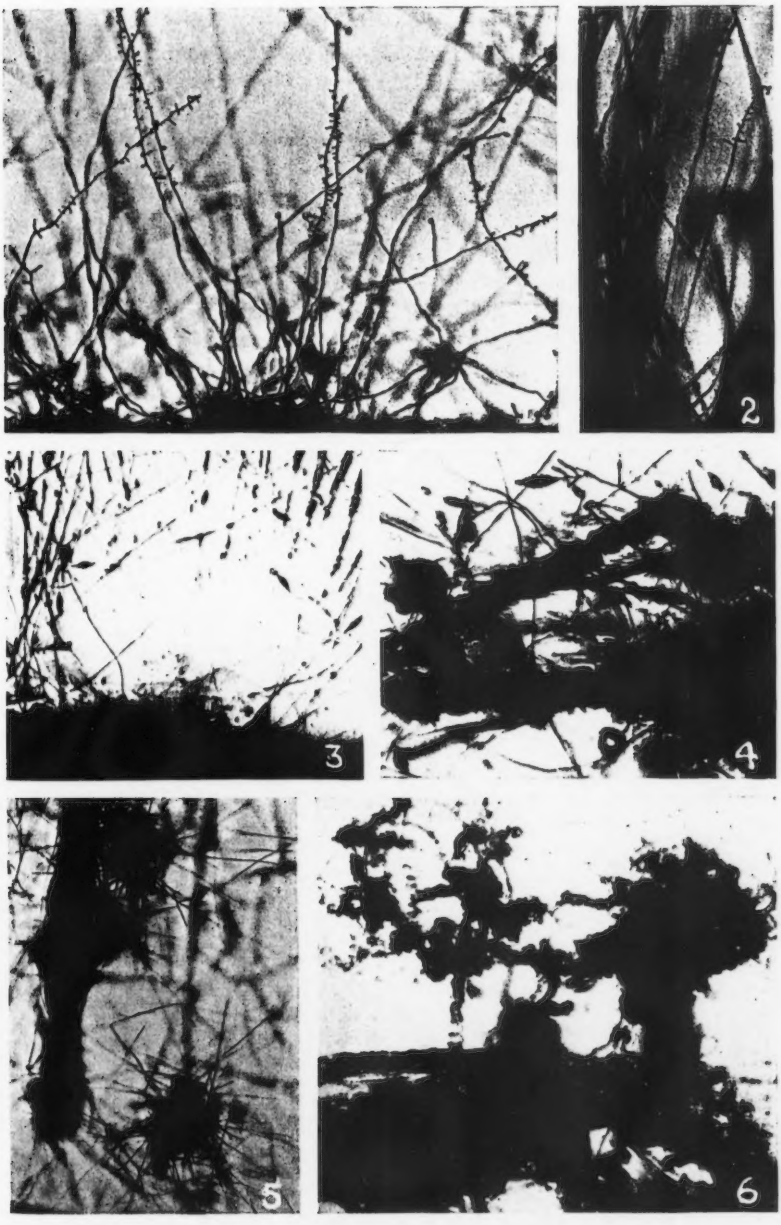
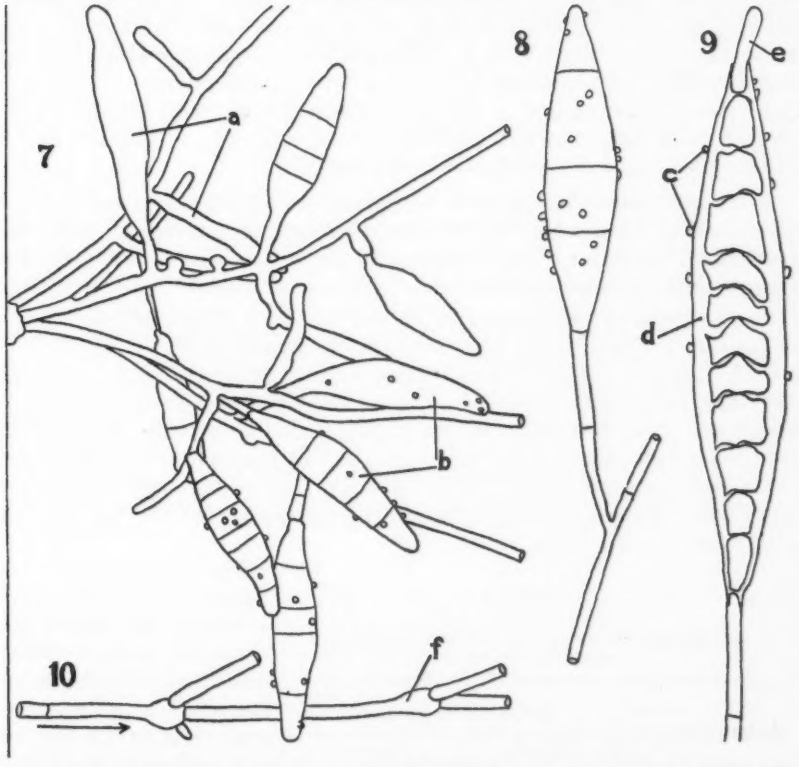


FIG. 1. *Microsporon audouini. Aleuriospores on aerial hyphae produced by infected human hair kept at 99% humidity (0.5 M), × 102.* FIG. 2. *Microsporon felineum. Aleuriospores on aerial hyphae from infected cat hairs kept at 99% humidity (0.5 M), × 102.* FIG. 3. *M. felineum. Fuseaux bearing apical drops of liquid, produced on mycelium from infected human hair kept in saturated atmosphere, × 47.* FIG. 4. *M. felineum. Fuseaux produced from infected cat hairs. The torulose surface is clearly visible, × 102.* FIG. 5. *Trichophyton gypseum. Developing spore bushes on mycelium produced from infected human hair kept in saturated atmosphere, × 61.* FIG. 6. *T. gypseum. Spirals produced from infected hair kept at 99% humidity (0.5 M), × 210.*

Microsporon felineum

Microsporon felineum Mewborn. *In situ* cultures: *aerial hyphae* straight, diameter 1–3.5 μ; *aleuriophores* (conidiophores), long, erect, usually unbranched; *aleuriospores* (conidia), sparse, hyaline, unicellular, borne singly and laterally, sessile or on short necks, pyriform to oblong, proximal end faceted, distal end round, 3–6 × 1.5–2 μ; *fuseaux* (macroconidia) abundant, terminal on short lateral branches of main hyphae, thick walled, hyaline or brownish, straight, fusiform, sparsely torulose, 3–11 septate, proximal end faceted, distal end round, 50–120 × 20–25 μ; *spirals* absent.

In a saturated atmosphere germination normally took place within 24 hr., but at lower humidities it was delayed (Table V). The germ tubes consisted of straight, even, relatively stout hyphae, which remained unbranched for some time. As in *M. audouini*, in saturated atmospheres, conspicuous drops



FIGS. 7–10. *Microsporon felineum. Infected-hair cultures.* FIG. 7. *Group of developing fuseaux as seen in air;* a, *young spores;* b, *older spores becoming torulose and septate.* FIG. 8. *Mature fuseau in air.* FIG. 9. *Mature fuseau in glycerine jelly:* c, *wart-like protuberances;* d, *region of greatest thickening of cell wall;* e, *germ tube emerging from apical cell.* FIG. 10. *Racket hyphae:* f, *swelling behind septum. Magnifications: Nos. 7 and 8,* × *485; Nos. 9 and 10,* × *1100. Humidities: No. 7, 98% (1.0 M); Nos. 8, 9 and 10, 100% (0.0 M).*

of liquid were excreted at the tips of the aerial hyphae. Sometimes the presence of these drops led to the production of loops in the mycelium, resembling those referred to by Sabouraud (8, p. 713) as pelotons. They appeared to be formed by the growing tip of the hypha being unable to escape, owing to surface tension, from the drop surrounding its tip and consequently becoming coiled during subsequent growth around the surface of the drop. These pseudo-spirals differ entirely from the true spiral structures which are met with in *Trichophyton gypseum* and are described below. Spirals, as observed by Langeron and Milochevitch (6) in cultures of *M. felineum* on oats, etc., have not been observed in infected-hair cultures.

The aerial hyphae were straight with uniform diameter which varied from 1 to 3.5 μ; the older hyphae were constricted slightly at the septa. The hyphae were septate at intervals of 20–60 μ. The main hyphae branched fairly freely. The majority of branches were formed just behind a septum and left the parent hypha at right angles but at once bent forward and came to lie at an acute angle with the parent hypha. Racket hyphae, in which branches arise from swellings behind the septa, characteristic of this species in culture were not found in the aerial mycelium, but were present on the glass cover of the cell in saturated atmospheres (Fig. 10). Abundant hyphal fusions were observed between aerial hyphae.

After some days aleuriospores were borne on long, straight, unbranched aerial hyphae (Plate 1, Fig. 2). In size and shape the aleuriospores resembled those of *M. audouini*. Their appearance in the infected-hair cultures was sporadic and much less frequent than fuseaux in this species.

Fuseaux were usually produced on the mycelium resulting from the infected-hair cultures at all humidities except those sufficiently dry to limit growth (Plate 1, Figs. 3 and 4). They were developed in from four to six days in a saturated atmosphere but were delayed considerably at the lower humidities. The occurrence of fuseaux was found to be somewhat sporadic and it was noticed that when the portion of hair placed in the cell was short no fuseaux were produced. Table II shows the relation observed in one experiment between the length of the infected portion of the hair and the occurrence of the fuseaux.

TABLE II

RELATION BETWEEN LENGTH OF INFECTED HAIR AND OCCURRENCE OF FUSEAUX

Length of hairs	Occurrence of fuseaux	
	Present	Absent
From 0.8 to 1.8	0 preparations	6 preparations
From 2.0 to 5.0	6 preparations	1 preparation

The fuseaux (Figs. 7-9) apparently represent modified lateral branches of the aerial hyphae. These modified branches consisted of one or two basal stalk cells which eventually collapsed, bearing a large, thick walled, spindle-shaped spore. Perforate septa divided the interior of the spore into from

about 4 to 12 cells. The length of the fuseaux varied from 50–120 μ, and the diameter from 20–25 μ. The size and degree of thickening of the wall was variable and appeared to depend upon the amount of infected material available to the developing mycelium. In some preparations the spores remained immature and thin walled and then somewhat resembled those of *Trichophyton gypseum*. The region of greatest thickening where the wall was sometimes 4 μ thick, coincided with the maximum girth of the spore. It was thinnest at the rounded apex, and at the cross wall of the hindmost cell where it had broken from the parent hypha. Germination usually took place through the thin wall of the apical cell and was sometimes observed while the fuseau was still attached to the parent hypha. The outside wall of the fuseaux was usually covered with minute colorless warty prominences which may be specially well developed on the apical cell. The mode of development of fuseaux and the origin of their septa as a ring-like ingrowth from the cell wall has been figured by Buller (2) from studies of infected-hair cultures made by one of the present writers.

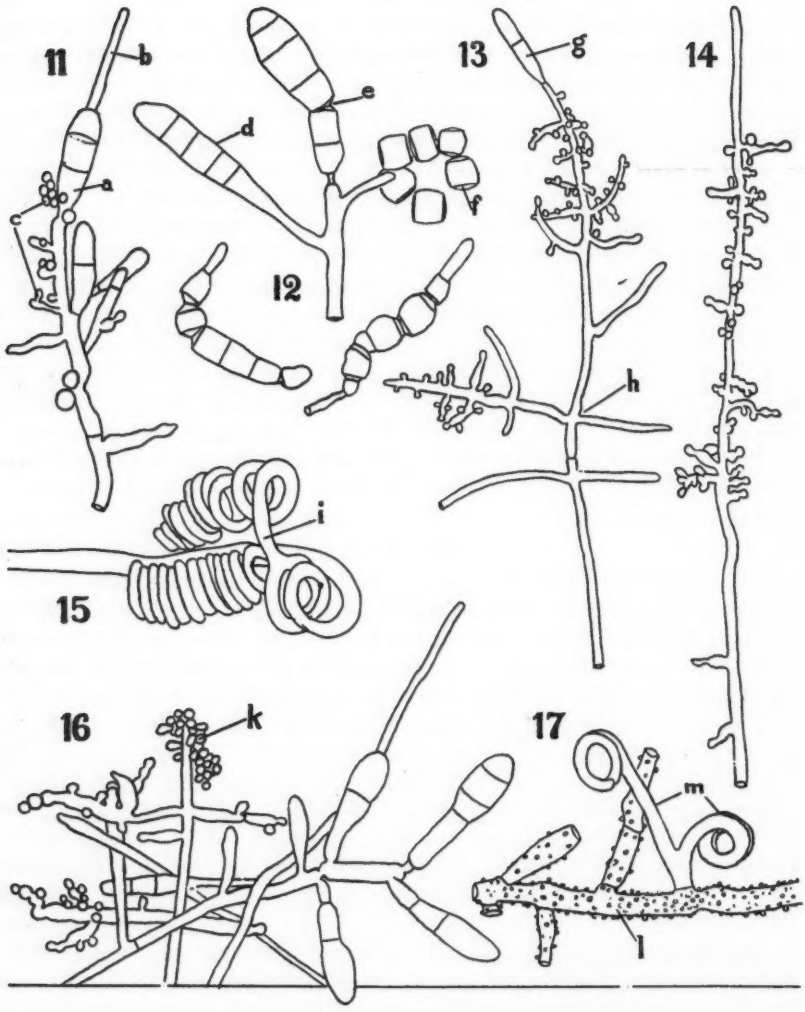
When the *in situ* cultures were transferred to Sabouraud's proof medium, typical mycelia of *M. felineum* were obtained.

Trichophyton gypseum

Trichophyton gypseum Bodin. *In situ* cultures: *aerial hyphae*, straight, diameter 1–3.5 μ, often cross branched; *aleuriophores* (conidiophores), long, erect, sparsely branched, or short much branched, and densely aggregated into white spore-bushes up to 1 mm. in diameter; *aleuriospores* (conidia) abundant, unicellular, hyaline, lateral sessile or terminal on lateral branches of hyphae, becoming densely aggregated by disintegration of parent hypha, proximal end faceted, distal end round, subspherical 2.8 $\times$ 2.5 μ, occasionally pyriform, 3–3.5 $\times$ 2.5 μ; *fuseaux* (macroconidia) often abundant, smooth, thin walled, hyaline, straight, clavate to cylindrical 4–10 septate, 40–75 $\times$ 7–10 μ, proximal end faceted, distal end round; *spirals* regular, counterclockwise, 1–30 turns, 8–12 μ in diameter, tightly or loosely coiled, sometimes branched, abundant in spore bushes; *torulose hyphae* present in spore bushes.

Germination of the fungus in hairs in a saturated atmosphere began within 24 hr. and was well advanced after 48 hr. For the first few days after germination the hyphae grew about 600 μ per day at a room temperature of 23° C. The mycelium consisted of unbranched straight hyphae varying from 1.0–3.5 μ in diameter, which did not show hyphal fusions until after the fourth day of growth when numerous fusions were observed in the aerial mycelium. Germination was slower at lower humidities and was not observed at humidities lower than 97% (1.5 *M*). The structures produced by the mycelium will be described at the humidity at which their development was most favorable.

The aerial hyphae branched at right angles to the parent hyphae, as did the species of Microsporon described above. A characteristic of *Trichophyton*



FIGS. 11–17. *Trichophyton gypseum. Infected-hair cultures.* FIG. 11. *Group of spores in air:* a, *fuseau and its germ tube* b; c, *aleuriospores.* FIG. 12. *Fuseaux six weeks old breaking down into chlamydospores;* d, *an intact spore;* e, *splitting of septum;* f, *fuseaux completely broken down.* FIG. 13. *Aleuriophore in air, with aleuriospores and small terminal fuseau; showing cross branching.* FIG. 14. *Aleuriophore in air.* FIG. 15. *Branched spiral from periphery of a spore bush, in glycerine jelly.* FIG. 16. *Sporogenous hyphae emerging from hair, showing mode of branching and position of fuseaux:* k, *group of aleuriospores collapsed on parent hypha.* FIG. 17. *Torulose hyphae bearing spirals,* m, *from interior of spore bush; mounted in glycerine jelly. Magnifications: Nos. 11, 12, 14 and 16,* × *485; No. 13,* × *315; Nos. 15 and 17,* ×*1100. Humidities: No. 11, 98% (1.0 M); Nos. 12 and 16, 99% (0.5 M); Nos. 13, 14, 15 and 17, 100% (0.0 M).*

gypseum, not observed in Microsporon, was the tendency for two rectangular branches to leave at opposite sides of the parent hypha. A characteristic rectangular appearance is then given to the mycelium (Figs. 13 and 16).

Unlike *Microsporon felineum*, in this species aleuriospores were produced regularly and in great numbers. But like the microsporons they were produced singly as round, unicellular, lateral bud-like outgrowths at right angles to the parent hyphae (Fig. 14), and were rounded at the distal end and faceted at the proximal end. They were smaller than the aleuriospores of the Microsporon species so far studied and more regularly nearly spherical. They varied from 2.8 to 3.5 μ in length by 2.5 μ in breadth and were almost sessile. The aleuriospores first appeared on the aerial mycelium about five days after germination had begun. In a saturated atmosphere they were at first borne at the ends of long delicate aleuriophores which differed from those of *M. audouini* and *M. felineum* in having a greater number of branches. Fig. 13 shows the tip of an aleuriophore bearing spores at a distance of one millimetre from the hair; the branches crossing the main hypha at right angles were characteristic of the mode of branching of the sporogenous hyphae of this species. The first formed aleuriospores were widely distributed in the aerial mycelium.

Later however the production of aleuriospores began to be concentrated into certain areas. At intervals in the mycelium, usually near the hair, dense bush-like masses of mycelium arose by the repeated rectangular branching of hyphae (Plate 1, Fig. 5). These no doubt corresponded to the so-called "sterile perithecia" of Langeron and Milochevitch (6). On the cover slips of the preparations they were visible to the naked eye as white points in the aerial mycelium. The hyphae composing these masses, which are here termed *spore bushes*, were stouter than the ordinary aerial mycelium, septate at shorter intervals, and branched in the usual rectangular manner of this species. In the interior of the spore bushes were produced great numbers of aleuriospores which, by the disintegration of some of the sporogenous hyphae, came to fill the space between the main hyphae of the structure without apparent order. No asci or ascospores have been observed at any stage of the spore bushes even after four months of growth in the cultures. Aleuriospores were most abundant at the higher humidities employed; under drier conditions fuseaux predominated. In the spore bush a rectangularly branched network of coarse torulose hyphae was often present (Fig. 17). Similar torulose hyphae were observed in this species in cultures on natural media by Langeron and Milochevitch. These hyphae did not bear spores but branched freely and frequently terminated in spirals. The warts appear to be of the same nature as the cell wall of the hypha, and do not resemble the collar-like projections left on a hypha after an aleuriospore has broken off.

Spirals usually appeared at about the same time as the spore bushes. At first they were scattered throughout the aerial mycelium, but later became more and more abundant and well developed around the spore bushes (Plate 1, Fig. 6). The spirals were in the form of three-dimensional, cylindrical

helices of from 1 to 30 turns, and consisted of very even, septate hyphae, about 1.5–2 μ in diameter, coiled into a spiral about 8–10 μ wide. The length of the spiral naturally depended on the number of turns and the distance between each successive turn of the coil. The spirals were very regular in form. They were counterclockwise structures, sometimes branched as illustrated in Fig. 15. As the structure and significance of these spirals will be considered in another communication, it is here sufficient to record that in the infected-tissue cultures they were abundant, exceptionally well developed, and exactly resembled those formed in culture on various media.

Fuseaux, unlike aleuriospores in this species, were more abundant when the humidity was low. Plate 2, Fig. 1, shows a naturally infected hair surrounded by a halo of fuseaux after six weeks at 98% humidity (1.0 *M*). The best development of fuseaux was observed at 98% humidity (1.0 *M*). The fuseaux of *T. gypseum* differed from those of *M. felineum* in shape and also in that the wall was relatively thin. They were club shaped bodies up to 70 μ long and 10 μ in diameter with the proximal end faceted. They were divided by perforate septa into from 4 to 10 cells and germination often took place through the apical cell while the spores were still attached to the parent hypha. The arrangement and relative sizes of the aleuriospores and small fuseaux is illustrated in Figs. 11 and 16. In cultures two months old fuseaux situated on the cover slip of the preparation were found to have undergone a secondary change. Each cell of the spore became enlarged, the septum between adjacent cells split and the cells separated as if by mutual pressure as shown in the drawing reproduced as Fig. 12. Thus each fuseau came to be divided up into a number of chlamydospores.

When infected hair cultures were transferred to Sabouraud's proof medium, characteristic powdery colonies of *T. gypseum* were obtained.

Achorion schoenleini

Achorion schoenleini (Lebert) Remak. *In situ* cultures: *aerial hyphae* sparse, erect, straight or sinuous, branching at right angles, or apparently dichotomous; *aleuriospores* (conidia) borne laterally on erect hyphae, hyaline, unicellular, sessile, pyriform, proximal end faceted, distal end round, 3–5 $\times$ 1.5–3 μ; *fuseaux* (macroconidia) clavate, poorly differentiated from mycelium; *spirals* absent.

When infected hairs from patients with favus were put in a saturated atmosphere germination occurred at only one or two points along the hair. Mycelial development was very poor, the farthest branches reached only about 100 μ from the hair. All the hyphae were confined to the film of water which at this humidity accumulated around the hair. The mycelium consisted of somewhat irregular, coarse, branched hyphae which soon formed cyst-like lateral and intercalary chlamydospores, (Fig. 18) and no further development was noted on favus hairs.

When, instead of hairs, scutula were placed in the moist chambers further development took place, perhaps in consequence of the greater supply of

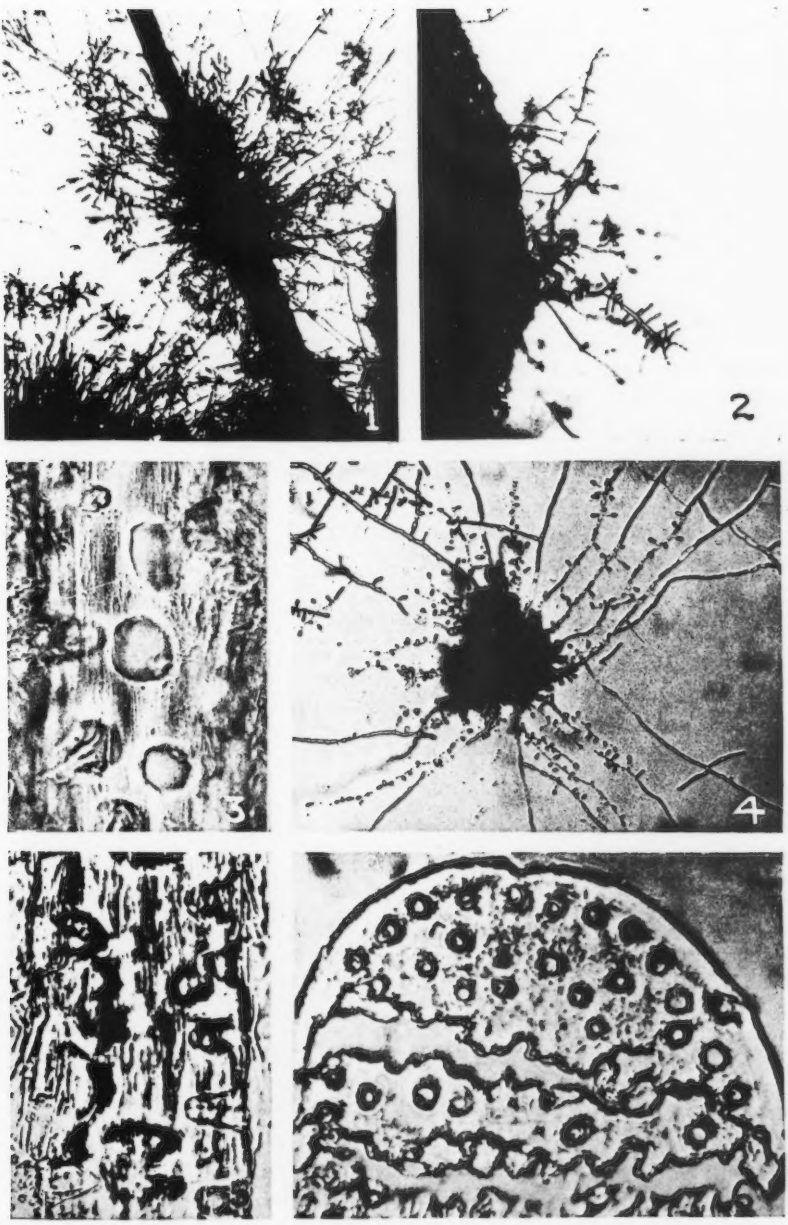


FIG. 1. *T. gypseum. Halo of fuseaux on mycelium growing from infected hair kept in saturated atmosphere,* × *45.* FIG. 2. *Achorion schoenleini. Aerial sporogenous hyphae developing from scutulum kept at 98% humidity (1.0 M),* × *102.* FIG. 3. *Saprophytic invasion of healthy human hair when placed for five days on surface of culture of Microsporon felineum on Sabouraud's agar. Surface of hair in focus, showing openings of wide rounded pits,* × *400.* FIG. 4. *A. schoenleini. Aleuriospores on hyphae adhering to glass cover of cell, developing from fragment of scutulum kept at 98% humidity,* × *150* FIG. 5. *The same as Fig. 3, but with axis of hair in focus, showing rounded bottoms of pits suggestive of enzymatic erosion,* × *400.* FIG. 6. *Transverse section human hair naturally infected with Achorion schoenleini, showing longitudinal canals in section,* ×*1050.*

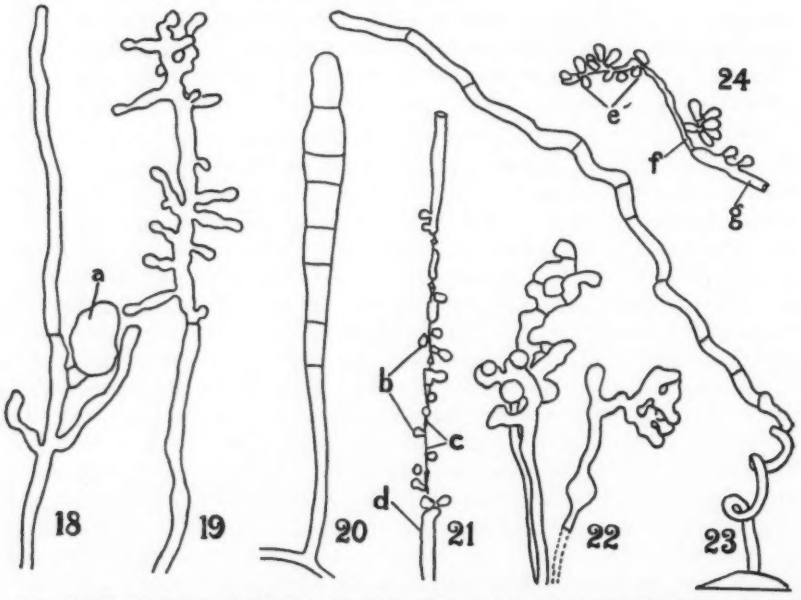
food present in a scutulum. In a saturated atmosphere (100% humidity (0.0 *M*)) some degree of bacterial contamination was always present, but it was never found to check completely the growth of the fungus from the scutulum. At this humidity the scutulum was always observed to accumulate around itself a considerable film of water, and all hyphae remained in the film. Germination began in about 24 hr. and after a few days the scutulum was surrounded by a fringe about 0.1 mm. broad of fairly straight, sparsely branched hyphae filled with granular protoplasm. The ends of the hyphae then underwent exceedingly irregular growth as illustrated in Fig. 22. The older parts of some of the hyphae then became emptied and terminal and intercalary chlamydospores were formed but no further development took place.

To obtain characteristic dermatophyte structures in *Achorion schoenleini* it was necessary to submit the scutula to considerably drier atmospheres. Germination was obtained in humidities as low as 93% (3.0 *M*) but the best growth was obtained at about 98% (1.0 *M*) at which humidity no bacteria developed and no free water was visible around the scutulum. Germination at 98% humidity (1.0 *M*) began in little more than a day and proceeded, as at 100% (0.0 *M*), until the scutulum was fringed with aerial hyphae. Extensive mycelium never developed in this species as it did in the dermatophytes referred to above, and to the naked eye the only visible change was a velvety appearance acquired by the scutulum. Some of the hyphae tended to spread over the cover slip in complex arborescent branching systems, which rendered observations on deeper lying structures difficult. It is remarkable that no hyphal fusions were observed in the mycelium of this species. In this respect *A. schoenleini* resembles *Trichophyton album* in cultures of which the writers have been unable to find hyphal fusions. On the other hand *A. schoenleini* differs from the other three species described in this paper which form abundant hyphal fusions in the aerial and submerged mycelium. As early as the fifth day after germination some of the aerial hyphae acquired rectangular cross-branches resembling those of the sporogenous hyphae in *T. gypseum* (Plate 2, Fig. 2; and Fig. 19). Aleuriospores were formed on these hyphae, and also on unbranched hyphae represented in Fig. 21. As in the three species described above the aleuriophore becomes emptied of protoplasm during the formation of spores as shown in Plate 2, Fig. 4; and Fig. 24. The aleuriospores were mostly pyriform rather than spherical, in this respect differing from those of *T. gypseum*.

Long club-shaped hyphae were common in the aerial mycelium, and these are regarded as fuseaux (Fig. 20). They were, however, rudimentary and not well differentiated from the ordinary mycelium. Thus scutulum cultures of *Achorion schoenleini* developed the characteristic dermatophyte fuseaux which were unknown until 1929 when Sabouraud and Negroni (9) observed them in cultures on a special medium containing honey. Sabouraud's belief that *Achorion schoenleini* is related to the more typical dermatophytes is thus supported by the occurrence in all four species of aleuriospores and fuseaux on the host tissue.

True spirals resembling those of *T. gypseum* were lacking, but a tendency to spiral twisting of the hyphae was of frequent occurrence in the aerial mycelium. One of these hyphae is illustrated in Fig. 23. Whether they have any relation to true spirals is doubtful.

When *in situ* cultures were transferred to Sabouraud's proof medium, typical mycelia of *A. schoenleini* were obtained.



FIGS. 18–24. *Achorion schoenleini. Scutulum cultures.* FIG. 18. *Vegetative hyphae produced in saturated atmosphere, with chlamydospore,* a, *on short lateral branch.* FIG. 19. *Aleuriophore drawn in air.* FIG. 20. *Rudimentary fuseau.* FIG. 21. *Part of sporogenous hypha;* b, *aleuriospores;* c, *remains of emptied hypha;* d, *living portion of hypha.* FIG. 22. *Portions of arborescent hyphae produced in saturated atmosphere.* FIG. 23. *Twisted hyphae.* FIG. 24. *Tip of aleuriophore:* e, *aleuriospores;* f, *collapsed portion of hypha;* g, *living hypha. Magnifications: Nos. 18, 20 and 22, × 485; Nos. 19, 21, 23 and 24, × 1100. Humidities: Nos. 18 and 22, 100% (0.0 M); Nos. 19, 21, 23, 24, 98% (1.0 M); No. 20, 96% (2.0 M).*

Viability of the Organisms

Moist cells were set up with hairs epilated recently and also with hairs stored for up to 28 months after removal from the patient. The parasite was found to remain dormant but viable for many months after detachment from the body. However, the specimens were stored under dry conditions in a laboratory where the relative humidity may fall as low as 10% during the winter, and material exposed to other atmospheric conditions in nature would not necessarily give the same results.

Microsporon audouini. Germination was obtained regularly from hairs stored for 12 and 15 months, but specimens tested after 27 months' storage failed to grow.

Microsporon felineum. Infected hairs removed from human patients and tested after 28 months did not germinate, but growth was observed from hairs removed from another patient 27 months previously.

Trichophyton gypseum. This organism was found to retain its viability in hairs for at least eight months after epilation.

Achorion schoenleini. Scutula from cases of favus capitis were viable for at least a year.

Incidentally it was noted that hairs infected by *Microsporon* which failed to germinate and in which the fungus was presumably dead, fluoresced in ultra-violet light as strongly as hairs recently epilated. It therefore appears that the life of the fungus is not a necessary factor in the phenomenon of fluorescence.

Effect of Various Humidities

Table III shows the relation between the molar concentration, osmotic pressure, and percentage saturation of the atmosphere in contact with the humidifying solutions, as calculated from data given in International Critical Tables (4) for temperatures from 20°–25° C. The 1% of copper sulphate contained in the solution is disregarded in these calculations which must be regarded as approximate only.

TABLE III

RELATION BETWEEN CONCENTRATION, HUMIDITY AND OSMOTIC PRESSURE

Conc. of humidifying soln., moles per litre	0.0	1.0	2.0	3.0
Humidity of atmosphere, % saturation	100.0	98.1	95.8	93.3
Osmotic pressure, atm.	0	26	51	77

The effects upon the development of the organisms of the various humidities to which the infected hairs and scales were exposed can be dealt with under the following headings: (i) effect on bacterial contamination; (ii) effect in limiting germination; (iii) effect upon the growth rate of the mycelium.

(i) *Bacterial contamination.* Contamination of the infected-tissue cultures by saprophytic molds occurred occasionally in cells at any of the humidities employed. Bacteria were seldom found in tissues infected by *Microsporon audouini* or *M. felineum.* Hairs infected by *Trichophyton gypseum* or scutula of *Achorion schoenleini* usually became contaminated by staphylococci during the first day or two if the atmosphere in the cell was nearly saturated with water vapor (100 to 99.5% humidity (0.0 to 0.3 *M*)). At humidities lower than these bacteria did not grow. It appears that the fungi are able to grow under much drier conditions than these bacteria, a fact which may be useful in isolating fungi from material which is bacterially contaminated. Of the four dermatophytes studied, that with the narrowest range of humidity tolerance grew over solutions of from 0.0 to 1.5 *M*, while the bacteria present were checked in the absence of free water. Often the cocci developing around the diseased material of *T. gypseum* were rapidly checked by the growth of the fungal hyphae, while the hyphae did not appear to be permanently affected by the contamination.

(ii) *Limiting germination.* Although the dermatophytes were capable of growing under considerably drier conditions than the bacteria encountered, a comparison of Tables III and IV will show that even for them a very high percentage of atmospheric moisture is necessary if they are able to imbibe enough water from the air to make germination and growth possible.

TABLE IV

HIGHEST CONCENTRATION OF HUMIDIFYING SOLUTION OVER WHICH GROWTH WAS OBSERVED

Species	Limiting concentration, molarity
Microsporon felineum	2.0
Microsporon audouini	1.5
Trichophyton gypseum	1.5
Achorion schoenleini	3.0

From a comparison of Tables III and IV it will be seen that the fungi present in infected tissue are able to imbibe water from the atmosphere over a humidity range of only about 5% below saturation. So long as the fungus is growing the fungus cell must have imbibition forces sufficiently great to remove water vapor from the atmosphere. At the lowest concentration of the humidifying solution over which the fungus is unable to imbibe water both fungus and solution must be in equilibrium with the water vapor in the atmosphere of the chamber. This is taken to indicate that the suction force of the fungus cells is greater than the osmotic pressure of the solution of the highest concentration over which growth is possible. This is a modification of the principle involved in Barger's well known method of comparing the osmotic pressures of two solutions. Such are the forces available to the fungus for drawing water out of the air below the saturation point or from the soil with which it is in contact.

(iii) *Rate of growth of the mycelium.* Radial growth of the mycelium was most rapid in cells containing atmospheres of the higher humidities. In cells with a saturated atmosphere, the hyphae became immersed in the drop of liquid which accumulated around the tissues, or else became irregularly branched owing to the surface tension of the liquid. Again at the higher humidities it was sometimes retarded by bacterial contaminants. Better growth was therefore often obtained at humidities slightly below saturation. In one experiment (Table V) careful measurements were made with the micrometer eyepiece of the daily growth at various humidities from hairs infected by *M. felineum.* Each humidity was represented by two preparations, and measurements were taken in two positions on each mycelium.

TABLE V

RADIAL GROWTH OF *Microsporon felineum* AT VARIOUS HUMIDITIES

Conc. of humidifying soln., *M*	0.0	0.5	1.0	1.5	2.0
Time of germination, days	1	1	1	2	4
Mean daily growth on 2nd and 3rd days, μ	1000	870	500	350	50*

**Only a few spores had germinated.*

Discussion

The life history of the four common dermatophytes described above appears to be divided into two phases which are termed for convenience *parasitic* and *saprophytic*, although even in the lesion on the body the milder ringworm pathogens, such as *Microsporon audouini*, do little more than attack tissue already dead and keratinized.

The parasitic phase is passed on the animal body, where the keratin of the epidermis, and possibly the lymph, provides the organism with nutriment. The phase on the animal body is characterized: (i) by the formation of immense numbers of thallospores, or in *Achorion schoenleini*, of compact masses of hyphae known as scutula; (ii) by the longitudinal direction of the hyphae within the hair; and (iii) by the fact that each hypha within the hair lies in a narrow longitudinal canal which it completely fills. A cross section of a hair infected by *Achorion schoenleini* (Plate 2, Fig. 6) shows the longitudinal canals cut through transversely.

The saprophytic phase has long been known in culture. The four species tested by the *in situ* culture method were found to produce all the spore forms and most of the mycelial forms hitherto known only in culture on various media. They are therefore able to pass both the parasitic and the saprophytic phases on the host tissue alone, without the necessity of supplementary nutriment. From these experiments the saprophytic phase is believed to be initiated in nature under suitable conditions of humidity when the infected tissues become detached from the animal lesion, or possibly, if the infected animal dies, upon the dead body.

The saprophytic mycelium produces typical aleuriospores and fuseaux. Growth in the saprophytic phase appears to take place principally by the imbibition of water by fungal protoplasm which was formed during the parasitic attack of the tissue, and little by colonization of those parts of the hair or scale which remained unutilized. Portions of the tissue which were unattacked when the preparations were set up frequently remained unattacked for several weeks after the production of aleuriospores. Eventually, however, some colonization of the unattacked keratin took place, especially when the moist cells contained a saturated atmosphere. This saprophytic colonization of the hair, as described below, results in a type of attack differing entirely from that which characterizes attack during the parasitic phase. The saprophytic phase of the dermatophytes studied thus resolves itself into two stages: local growth resulting from imbibition of water by fungal protoplasm formed during the parasitic phase; and growth by colonization of fresh tissue. The characters of the two stages will be considered separately.

The stage of saprophytic growth by imbibition is characterized in *Microsporon audouini*, *M. felineum* and *Trichophyton gypseum*, by the germination of spores in the spore sheath and the establishment of hyphal fusions between the resulting hyphae, producing a radiating, branched, sporulating mycelium which may have a diameter of 1 cm. or more. During this stage the tissue previously infected is more thoroughly penetrated by hyphae. Growth of

the scutulum of *Achorion schoenleini* may be likened to the germination of a sclerotium, which becomes surrounded with a fringe of short sporogenous hyphae.

The stage of saprophytic colonization is characterized by: (i) the erosion of the hair in a radial direction by hyphae which lie in wide pits which they do not fill; (ii) by the rounded ends of these pits which strongly suggest that the keratin around the hyphae has been dissolved by enzymatic action; and (iii) the subsequent development of aleuriospores on the newly colonized portion of the hair. Exactly similar rounded pits were produced when uninfected hairs were laid upon the surface of living cultures of *Microsporon felincum* as shown in Plate 2, Figs. 3 and 5. When uninfected hairs in moist test tubes were inoculated with *Trichophyton gypseum* the same type of wide rounded pits was produced leading eventually to the more or less entire decomposition of the hair on which in turn were developed aleuriospores, fuseaux and spirals. The factors which determine the differences in the mode of attack upon the hair during the parasitic and saprophytic phases are at present unknown, but it is suggested that they are to be looked for in the highly specialized environment provided by the animal body, rather than in changes in the nuclear phase of the parasite. Asci have not been observed in infected-tissue cultures, even when preserved for six or eight months.

In nature the saprophytic phase is believed to be passed on detached host tissue. One fact which supports this hypothesis is that very few organisms are able to attack keratin. Considerations which support the hypothesis were discussed in the writers' preliminary note (3). "Although ringworm fungi will grow upon various sterilised natural media, such as horse-dung, straw, and grains of cereals, it has not yet been shown that they can flourish on these substrata under natural conditions which involve competition with other organisms. In the fallen infected hairs, on the other hand, a dermatophyte is in an advantageous position relatively to other organisms. By the time the infected hairs and scales fall from the body, the parasite has thoroughly penetrated their substance and converted much of it into fungal spores. A considerable amount of fungal protoplasm is therefore lying dormant within the hair ready to begin growth at once when brought into a suitable environment. Further, a fungus growing on such keratinised tissues as hair and scales has the advantage of freedom from competition, since it is growing on a substance which few other organisms are able to attack. It is possible that saprophytic stages of ringworm fungi may one day be found in Nature on various substrata other than fallen hairs. However, it may well be that cast-off hairs and epidermal scales are the most important natural substrata on which ringworm fungi pass their saprophytic existence and produce spores capable of infecting new human and animal hosts." Such a saprophytic phase in the life history of the dermatophytes must be of significance in the epidemiology of the diseases which they cause, especially in view of their long viability in infected tissue and ability to grow under drier conditions than bacteria.

The importance of the study of the microscopic structure of the dermatophytes must be emphasized. In the past, species have been based upon the gross appearance of the cultures on Sabouraud's media, especially upon texture, color and contour on these media. The microscopic organs have been believed to be variable and practically no attempt has been made to base species upon intimate studies of their morphology.

Naturally infected host tissue provides a relatively standard culture medium, upon which, under suitable conditions, mycelium aleuriospores, fuseaux and spirals develop. All the dermatophytes described above produce characteristic microscopic structures on host tissue by which the species can be distinguished. It is believed that when greater attention is paid to these structures the gross appearance of the colony will take its place as one of the several groups of characters by which these organisms may be classified.

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NOTES ON CERCARIAL DERMATITIS WITH DESCRIPTIONS OF THE CAUSATIVE ORGANISMS, *CERCARIA WARDLEI* N.SP., *CERCARIA BAJKOVI* N.SP., AND THE PARTHENOGENIC STAGE OF *CERCARIA ELVAE* MILLER[1]

By J. A. McLeod[2]

Abstract

This paper records an investigation of cercarial dermatitis, commonly known as *swimmer's itch* or *slough itch*, observed on bathers in Clear Lake, Manitoba. The infestation is manifest by the presence of erythematous papules and causes severe itching.

As a result of a survey for animal parasites in the lake, and experimental infestations with all forms it has been found that the disease may be caused by *Cercaria elvae* Miller, a brevifurcate distome cercaria, or by either of two pharyngeal longifurcate distome cercariae, *Cercaria wardlei* n. sp. and *Cercaria bajkovi* n. sp. Clinical and pathological notes on the disease are given.

Introduction

During the past 20 years numerous medical practitioners in Manitoba have been called upon to diagnose and prescribe treatment for a severe and unusual type of dermatitis which appears in the form of large erythematous papules which are intensely itchy. The fact that nothing of the nature of a bacterium could be cultured from extracts of these papules, and the observations that it is non-contagious and disappears spontaneously after a course of a few weeks, left considerable doubt as to the origin of the disease. In each case enquiry revealed that the affliction first made its appearance shortly after the patient had bathed in a body of water known as Clear Lake.

Numerous theories have been advanced regarding the origin of the disease. Some investigators have attributed it to the penetration of the pores of the skin by fine particles of sand. Others believed that it resulted from contact with poisonous plants growing on the shore, and some ascribed it to extreme individual susceptibility to toxic plant juices in solution in the water. Because of the failure of the disease to respond readily to treatment, such supposedly susceptible people were advised to refrain from further bathing in these waters.

Cases were not sufficiently frequent to warrant an extensive investigation until 1930 when the area surrounding the lake was converted into a national park. The increased number of campers and tourists visiting this resort resulted in an alarming number of cases of dermatitis. During the summer of 1933 over 55,000 people visited the lake and 50% or more of the bathers contracted the disease.

Past efforts on the part of two men, over a period of three years, to identify the causative agent of the disease have revealed nothing save that a fork-

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Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada.

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tailed organism was found in the water. Treatment of the water with large quantities of copper sulphate did not decrease to any extent the number of infestations.

The purpose of the investigation reported here therefore was to identify the causative organism and to determine the pathological condition produced and the duration of infestation. Knowledge of the life cycle with a view to bringing about efficient eradication of the parasite was also desirable.

An examination of the literature reveals the report of a similar disease by Cort (1). In this case *C. elvae* Miller was the causative organism and accidental infestation was produced by dipping the forearm into a pail of water containing parasitized molluscs. Further notes by the same author (2) state that a similar disease had been encountered in various parts of the United States, Haiti and France. In 1930 Matheson (4) reported cases from Cardiff in which *C. elvae* Miller was suspected. Similarly Taylor and Baylis (8) published the results of experimental work in Great Britain on a Cercaria which causes dermatitis. Again in 1930 Vogel (9) gave an account of cercarial dermatitis in Germany.

Procedure

Clear Lake, Riding Mountain National Park, is a body of exceedingly clear water about eight miles in length with a maximum width of three miles. The greatest known depth is 180 ft. and the bottom is of pure sand and coarse stones. Temperatures in June and July vary from a minimum of 0.9° C. in the deepest part to 29.5° C. in shallow water. Macroscopic aquatic vegetation is sparse and confined to localized patches near the shore.

A careful survey of the water in the vicinity of the bathing beach was made by means of fine plankton nets towed at various depths. Lower layers failed to yield any suspicious organism but the surface layer of about one foot in depth, on microscopic examination, produced the three species of furcocercous Cercariae listed below. Only living specimens were of any value for purposes of identification as contraction during fixation obliterated internal detail. Living specimens were lightly stained with dilute neutral red which penetrated the digestive tract and penetration glands. One minim of saturated chloretone in 30% alcohol added to 10 cc. of water containing the organisms was sufficient to anaesthetize active specimens without producing disintegration. Permanent preparations were made by fixing in hot 70% alcohol and mounting in glycerol jelly.

Eight species of gastropod molluscs were taken from these waters in an effort to determine the secondary host of the *Trematoda*. They may be briefly listed as follows:—*Limnaea stagnalis oppressa* Say.; *Limnaea obrussa* Say.; *Planorbis campanulatus* Say.; *Physa gyrina* Say.; *Volvata tricarinata* Say.; *Amnicola* sp., *Planorbis* sp.; *Planorbis trivolvis* Say.

Of these, only *Limnaea obrussa* and *Physa gyrina* were found to be infested. However both species are very plentiful over the entire lake from a foot above the water's edge to a depth of 4–5 ft. where they are found clinging

to the stones and vegetation. Large numbers of gastropods were gathered at different times for the purpose of determining the percentage of infestation. This was done by simply isolating each snail overnight in a test tube of water, which was then examined for cercariae, or by dissection of the snails. The results showed that 87% of all snails were parasitized.

The actual escape of cercariae from the mollusc host has not been observed closely but the rate is somewhat greater in darkness than in the light. Eight snails placed in 500 cc. of pure water for 14 hr. in darkness liberated 15,875 cercariae. These organisms are both phototactic and thermotactic and tend to congregate near the surface of the water or, if in a beaker, they gather at the side toward the light. If a finger be placed in a beaker containing cercariae, under a microscope the Trematoda will be seen to travel immediately towards the finger and repeatedly dart toward its surface in an effort to find an opening or grasp a hair.

Multiple infestations of molluscs were commonly observed during the survey but usually only a single species was obtained from the snails of one area. These areas varied in width from a few yards to several hundred, with an uninfested area between in some cases and overlapping in others.

All attempts to determine the primary hosts or obtain the adult stage of the parasites gave negative results. Five young and two adult white mice were placed in water containing all three species of cercariae for periods varying from 30 to 60 min. Post-mortem examinations of these one month after exposure failed to reveal any adult trematodes, although other investigators (6, 7) have been successful in producing infestations with other species.

Clear Lake is visited by a great many species of water birds but it was impossible to obtain any of them for examination. Post-mortem examination of four specimens of *Citellus tridecemlineatus* and one short-tailed shrew, *Balarina brevicauda talpoides*, showed them to be free from trematodes. Examination of the feces of specimens of *Lepus americanus* and *Fiber zibithicus* also from the shores of the lake gave negative results.

Taxonomy

The lack of any knowledge of the adult stage of the two previously unrecorded species necessitates the use of the larval name "*Cercaria*."

Classification of the species, based on the plan of Miller (5), is as follows:—

Pharyngeal brevifurcate distome cercaria, Group D:—*Cercaria elvae* Miller.

Pharyngeal longifurcate distome cercaria:—*Cercaria wardlei* n. sp.* and *Cercaria bajkovi* n. sp.**

Cercaria wardlei n. sp.

Type host. *Limnaea obrussa.*

Type locality. Clear Lake, Manitoba.

Specific diagnosis. A fairly large longifurcate pharyngeal distome cercaria about 770μ in length with a stout rectangular body. Furcae are slightly

*Named in honor of Professor R. A. Wardle, University of Manitoba.
**Named in honor of Dr. A. D. Bajkov.

longer than the tail stem and diverged while in the resting position. Anterior one-third of the tail stem and body is sharply turned to the left at an angle of 45° when the organism is suspended in the water. Three pairs of penetration glands are present posterior to the ventral sucker, which is one-third of the body length from the posterior end. The esophagus branches into two blind caeca which lie in the posterior body region. The anterior organ and ventral sucker bear numerous spines but an oral tuft of bristles is absent. Six pairs of flame cells are present in the body and one pair in the tail stem. Branches of the excretory tube open anteriorly in the mid-furcal region.

The average dimensions of moderately extended living specimens under a cover glass are:—body, 180 by 41μ; tail stem, 284 by 36μ; furcae, 306μ. Those of well extended fixed specimens in glycerol jelly are:—body, 144 by 27μ; tail stem, 274 by 27μ; furcae, 262μ. The anterior organ is one-fourth as long as the body, pyriform with the small end directed inward; two apparent head glands are present. The ventral sucker is a relatively large circular structure one-third of the body length from the posterior end and is slightly protrusible. The anterior organ and ventral sucker bear numerous retrorse spines and the anterior portion of the body is lightly spined. The tail stem is attached slightly ventrad to the posterior body termination; its surface is finely annulated and definite caudal glands are absent but it contains numerous small caudal bodies. From the periphery to a central fibre run numerous heavy bands of striated muscle in an oblique direction.

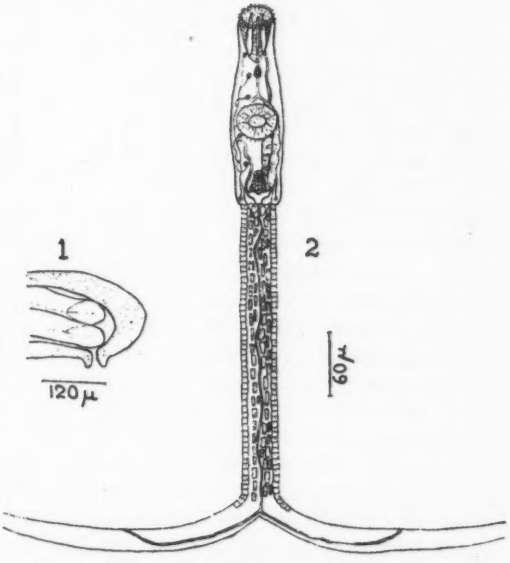


FIG. 1. *Cercaria wardlei, sporocyst showing birth pore.*
FIG. 2. *Cercaria wardlei showing internal structure.*

The mouth opening is subterminal; a pharynx is present around the narrow esophagus which bifurcates in the mid-body region into two large club-shaped caeca. These pass to within a short distance of the posterior end of the body. Three pairs of coarsely granular penetration glands are located posteriorly in the body between the germ cell mass and the ventral sucker. Their ducts are quite conspicuous and open dorsally and laterally to the mouth opening. Six pairs of flame cells are present in the body, disposed in each lateral half into an anterior and a posterior group of three, and

one pair in the tail stem. Branches of the excretory tube open anteriorly in the midfurcal region.

The parthenitae are long unbranched slightly motile sporocysts with almost uniform diameters of 170μ. Each contains numerous cercariae in various stages of development. The terminations are bluntly rounded, the birth pore being subterminal with conspicuous lips.

This larva may be readily distinguished from the other two species usually found associated with it by the resting position assumed in the water. Violent vibrations of the body and tail, with the anterior and posterior ends of the tail stem as fixed points, produce erratic dashes lasting 10–15 sec., the tail end preceding the body. These movements are followed by longer periods of resting in which the organism floats furcae upward and bent at an angle of 60–80° just below the water surface. The tail stem is almost vertical in position with the anterior one-third and the body turned sharply to the left at an angle of 45°. Movement under a cover glass is produced by similar vibrations or in an anterior direction by alternate attachment of the anterior organ and the ventral sucker. Form changes under a cover glass are very numerous, a spindle shape commonly being assumed by the body.

The above-described Cercaria bears a close resemblance to *C. chrysenterica* Miller in external appearance and in the position of the caeca and penetration glands. Major differences exist however in the number and arrangement of the parts of the excretory system. Also definite caudal glands are present in *C. chrysenterica* and absent in this species. It bears even a closer resemblance to *C. flexicauda* Cort and Brooks (3) in the shape of the organism while in the resting position. Slight metromorphic differences exist between the two species. The flame cells differ in the tail stems of the two species and *C. wardlei* has three pairs of penetration glands while *C. flexicauda* has only two. The oral tuft of bristles so conspicuous in *C. flexicauda* is absent in the above species. The writer is of the opinion that this larva has not been previously recorded and the name *Cercaria wardlei* is suggested.

Cercaria bajkovi n.sp.

Type host: *Limnaea obrussa* and *Physa gyrina*

Type locality: Clear Lake, Manitoba

Specific diagnosis. A slender-bodied pharyngeal longifurcate distome cercaria measuring about 600μ in living specimens. The body and tail stem are of about equal length but each is considerably shorter than the furcae. The body and tail stem are in a straight vertical line and the furcae diverge at an angle of 45–60° when the organism is resting suspended in the water. Two pairs of penetration glands are present anterior to the ventral sucker, which is situated one-fourth of the body length from the posterior end. The esophagus branches into two blind caeca which end in the posterior body region. The anterior organ and ventral sucker bear numerous spines but an oral tuft of bristles is absent and the body is smooth. Four pairs of flame

cells are present in the body, disposed in each lateral half, into an anterior and a posterior group of two. One pair of flame cells is present in the tail stem, and branches of the excretory tube open posteriorly in the midfurcal region.

The average dimensions of moderately extended living specimens under a cover glass are:—body, 189 by 45μ; tail stem, 180 by 32μ; furcae, 228μ. Those of well extended fixed specimens are:—body, 158 by 28μ; tail stem, 135 by 27μ; furcae, 144μ. The anterior organ is about one-fourth as long as the body and is very protrusible and spindle-shaped, the posterior end being sharply pointed. The ventral sucker is circular, about 21μ in diameter and situated about one-quarter of the body length from the posterior end. It is only slightly protrusible. Oral cap and ventral sucker bear numerous retrorse spines. The circumoral spines are long and during the extensive protrusion and retraction of the anterior organ point first forward and then backward. They evidently are of considerable importance in penetration. The tail stem is terminally attached and its surface is finely annulated. About six pairs of caudal glands are present with what appear to be fine ducts leading to the exterior.

The mouth opening is subterminal; a pharynx is present around the narrow esophagus which bifurcates in the mid-body region into two club-shaped caeca. These caeca pass to a position posterior to the ventral sucker. Two pairs of granular penetration glands are located in the body lateral to the esophagus and anterior to the ventral sucker. Their ducts pass anteriorly and open lateral and dorsal to the mouth opening. Four pairs of flame cells are present in the body, disposed in each lateral half into an anterior and a posterior group of two. One pair of flame cells is found in the tail stem, the left being farther posterior than the right. Branches of the caudal excretory tube open posteriorly in the midfurcal region. Striated muscle is present in the tail stem in the form of two heavy lateral bands running from end to end.

The parthenitae are long, branched, motile sporocysts containing a large number of developing cercariae. The diameter is fairly uniform and of about 126μ. The terminations are sharply pointed and the birth pore is subterminal without lips.

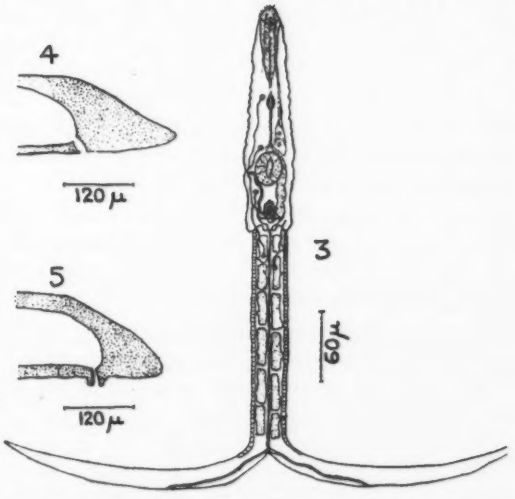


FIG. 3. *Cercaria bajkovi showing internal structure.* FIG. 4. *Cercaria bajkovi, sporocyst showing birth pore.* FIG. 5. *Cercaria elvae, sporocyst showing birth pore.*

Locomotion by this organism in the water is brought about by violent vibrations of the body and tail, the direction of motion being posterior. These periodic dashes are followed by longer resting periods during which the body and tail stem hang exactly perpendicular in the water and the furcae diverge at an angle of 45–60° just below the surface. Locomotion under a cover glass is produced in a similar manner, but creeping in an anterior direction is very limited owing to the inability of the anterior organ to become attached to a flat surface. Great extension of the body is possible and is followed by a flowing of the organs to the anterior end and a drawing up of the posterior body region and tail. During extension the sides of the body are almost parallel and annulated in the anterior half. The anterior end is pointed and the posterior somewhat concave.

C. tenuis Miller bears a greater resemblance to the above-described cercaria than any other recorded species. The distinction between the two is based on the difference in host specificity and marked differences in the arrangement of the excretory organs. The tuft of oral bristles so prominent in *C. tenuis* is absent in the above. These, in the opinion of the writer are sufficient grounds to warrant the creation of a new species for which the name *Cercaria bajkovi* is suggested.

Cercaria elvae Miller 1923

Host. *Limnaea obrussa*

Locality. Clear Lake, Manitoba

A brevifurcate distome cercaria measuring, in living specimens, as follows:—body, 306 by 75μ; tail stem, 360 by 39μ; furcae, 270μ. The spindle-shaped body tapers more gradually from the ventral sucker toward the anterior end than toward the posterior. The ventral sucker is very protrusible and bears two sets of muscle fibres. The anterior organ is large, being 63μ in length. The alimentary canal is a slender tube which bifurcates into two short caeca just anterior to the ventral sucker. Two prominent eyespots are present on the dorsal side and are directly connected with a bilobed mass of nerve tissue which lies just ventral to them.

Five pairs of penetration glands are present and are arranged in two groups, there being two pairs of circumacetabular glands and three pairs posterior to the ventral sucker. The ducts of these glands are prominent and open anterior and lateral to the mouth opening which is subterminal and ventral.

Seven pairs of flame cells are present in the excretory system. Three are anterior to the ventral sucker and empty into the anterior collecting tubule, while three are posterior and empty into the posterior collecting tubule. A seventh pair is present in the proximal region of the tail. The bifurcations of the caudal excretory tubule open at the tips of the furcae.

The parthinetae have not to the writer's knowledge been recorded but were found to be in the form of much branched sporocysts each having a uniform diameter of about 132μ. Each contains large numbers of developing cercariae. The terminations are sharply pointed; the birth pore is subterminal, closely resembling that of *C. bajkovi* but with prominent lips.

The body measurements of this organism are slightly less than those given for *C. elvae* Miller. However the relative body measurements and the arrangement of the body organs are identical. The shape and location of the eyespots and the number and position of the flame cells indicate that the above organism may correctly be considered as *C. elvae*.

Clinical Notes and Pathogenicity

The greatest number of infestations are found among people who bathe in water six feet or less in depth, as the parasites and their mollusc hosts are almost entirely confined to this region. The organisms are much more plentiful in the water during or directly after dull rough weather, since their liberation is much more rapid in the absence of bright light. About one person out of six appears to be immune to this disease. Women and children are more susceptible than men, although the disease is quite common among men. The majority are reinfected each time they enter the water, since an immunity, if ever acquired, requires considerable time.

Almost immediately upon leaving the water a tickling sensation of the skin is experienced in the infected areas. This continues and gradually develops into a severe itching in about four hours. Pronounced urticaria-like papules with whitish centres and highly inflamed margins appear 12 hr. after infection (Plate I, Fig. 1). Infection is usually much heavier on the arms and legs, as the bathing garments afford some protection to the rest of the body. The papules may be found in groups, or irregularly dispersed in varying numbers. The heaviest infestation observed consisted of 64 papules on the lower limbs of a three-year-old child. The dimensions of the papules vary with the individual from 5 to 15 mm. in diameter and from 1 to 4 mm. high. A hair follicle is usually present in the central elevation.

After 24 hr. the papules are highly inflamed and of a uniform reddish color. The itching is severe. Pus is never present unless secondary bacterial infection is produced by scratching, but in 36 hr. edema is evident and a drop of straw-colored exudate may be squeezed from each papule. Itching and inflammation usually disappear in five days with the regeneration of tissue beginning and the formation of a crust at the site of the papule. In the majority of cases only red macules remain at the end of three weeks.

The penetration of the skin by *C. wardlei* was observed on several occasions. A drop of water containing numerous organisms was placed on the writer's forearm under a binocular. The organisms darted actively about for a time until one of the suckers became attached to a hair. By grasping the hair with the ventral sucker and anterior organ alternately, the organism reached the surface of the skin. The anterior organ was then inserted into the cavity of the hair follicle and by a creeping movement of the body and lashing of the tail, penetration was rapidly effected. When the posterior end of the body reached the surface of the skin the tail broke off and floated away.

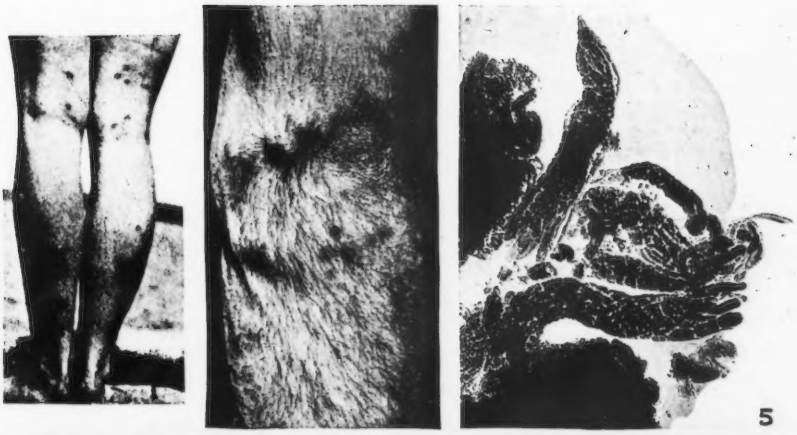
The method of locomotion after the skin has been penetrated is not known but an analogous phenomenon has been observed when the organisms were closely confined under a cover glass. Under this condition the anterior end sways slowly from side to side and repeated flowing of the body fluid and organs to the anterior end, with a drawing up of the posterior end, results in an amoeboid-like movement. The penetration glands are active at this time and a copious secretion of colloid-like substance is discharged from the ducts.

By means of this secretion the organism is able to cytolyze the tissue and migrate from the cavity of the hair follicle to the underlying tela subcutanea. Deeper in the subcutaneous tissue a large area surrounding the organism is broken down and an accumulation of fluid swells the space left by the cytolyzed cells (Plate 1, Fig. 3). Dissolving of the intercellular substance appears to take place for a considerable distance around the path of the organism with a loosening of the cells and fibres. Large numbers of leucocytes are to be found in masses in the tissue (Plate I, Fig. 4). Whether the organism eventually penetrates a blood vessel and is carried away to remain indefinitely as a hemophilic parasite, or is killed by the resistant forces of the body, is purely a matter of speculation.

All three organisms are capable of causing dermatitis and typical infestations were produced in each case by the author by simply immersing the forearm in water containing a single species of cercaria (Plate I, Fig. 2). Penetration by the organisms is more rapid when the arm is repeatedly dipped into the water and allowed to become almost dry before being returned, than when it is held submerged for the same length of time. Fifteen minutes is a sufficient time of exposure to produce an infection. It appears that the surface tension of the water film assists the organisms in gaining an entrance.

Acknowledgments

This investigation was carried out under the direction of Professor R. A. Wardle, Department of Zoology, University of Manitoba, to whom the writer wishes to express his sincere thanks for much valuable assistance and for the loan of apparatus. Thanks are also due Professor Ferris Neave and Dr. A. D. Bajkov, University of Manitoba, and Mr. C. S. Cunningham, Commissioner of Game and Fisheries, Province of Manitoba. Excision of the tissue shown in Figs. 3 and 4, Plate I, was possible through the courtesy of Dr. Geo. Bedford and Dr. C. E. Corrigan.



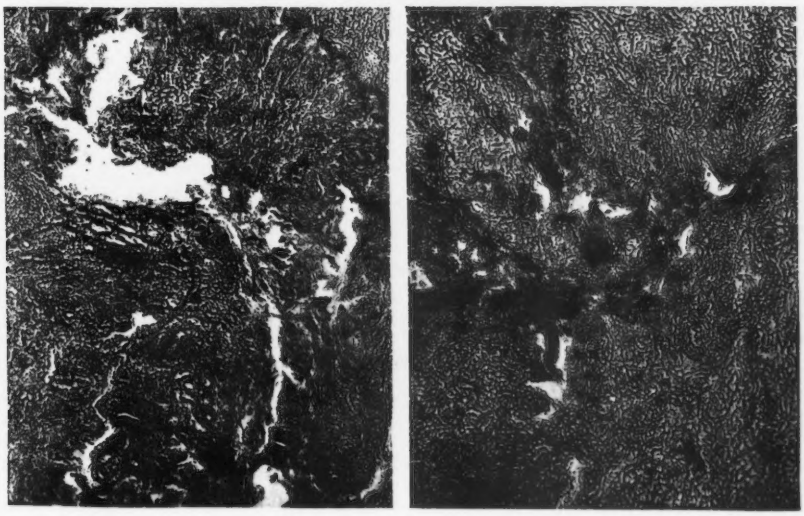


FIG. 1. *Typical infestation on the legs of a bather 12 hr. after exposure.* FIG. 2. *Papules on the writer's forearm 28 hr. after experimental infection with C. wardlei.* FIG. 3. *Photomicrograph of section of typical papule excised from writer's arm, showing path of migration of C. wardlei and loosening of the tissue elements.* ×50. FIG. 4. *Photomicrograph of section of same papule showing edema with masses of leucocytes.* ×50. FIG. 5. *Cercaria bajkovi. Photomicrograph showing sporocyst teased from digestive gland of Limnaea obrussa.* ×50.

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FLUKE DISEASE IN NORTHERN MANITOBA SLEDGE DOGS[1]

By J. A. Allen[2] and R. A. Wardle[3]

Abstract

An opisthorchiid Trematode, *Parametorchis manitobensis* n.sp. is recorded from the livers of sledge dogs from northern Manitoba. A key is given to the known species of *Parametorchis*. Infection with the trematode produces chronic cirrhosis and anemia, followed by muscular weakness and death. Infection is believed to be contracted by ingestion of metacercariae in *Catostomus commersonii* and prophylaxis would consist of the thorough boiling of such fish before use as dog food.

Introduction

While serving as a member of the expeditionary party, formed by Dr. R. G. Green in the summer of 1933 to study pathological conditions in grouse and wild rabbits of northern Manitoba, one of the writers (Allen) discovered the presence of numerous liver flukes in sledge dogs. The trapper who submitted the carcasses for autopsy, reported that nearly all his young dogs had died from an obscure disease and that he and other trappers in the vicinity of Mile 42 on the Hudson's Bay Railway (Cormorant Lake) were finding it increasingly difficult to raise dogs.

The existence of such insidious parasitic infestation in the dogs of these isolated regions is of great significance to trappers and others who are dependent upon sledge transportation during many months of the year. Dogs north of the 53rd parallel serve the inhabitants very much as horses did in settled districts 40 years ago, and a well conditioned team of huskies often takes the wayfarer through many a hazard.

In recent years a number of reports concerning heavy mortality in sledge dogs have come out of the North Country. Some of these reports and descriptions would indicate that an infectious, distemper-like disease was responsible for the mortality, but the lay mind is prone to confusion in matters pertaining to disease and pathology, and some of the outbreaks, perhaps, may be attributable to fluke disease and other forms of parasitism.

Further research in northern territories, where raw fish is the common diet of canines, will probably reveal that liver fluke disease is quite rampant. It is interesting to note here that Dr. J. K. Morrow, a practicing veterinarian of Winnipeg, found a fairly heavy liver fluke infestation in the cadaver of a husky dog brought to him for post-mortem examination on January 9, 1934. This subject was a member of a team of dogs which had travelled from the town of The Pas, located just 42 miles south of the district in which the writers' first cases were observed. Furthermore, this trematode proved to be of the same species as that found in the writers' cases. Fecal tests were

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made by the flotation and centrifugal methods on the seven remaining dogs of this team, and five of them were found to be lightly involved, only two or three ova being found in each smear.

Symptomatology

All the cases observed in the writers' brief clinical study had reached a stage of chronicity and consequently the manifestations of early infection could not be recorded. It is inconceivable, however, that there could be heavy invasion by a devastating parasite like the liver fluke without concomitant systemic reaction in the early stages. However, as is the case in most forms of internal parasitism, light infections may go unnoticed until the fecal test is applied. There is always the quantitative factor.

Progressive weakness is characteristic of the malady. The affected animal tires easily and lags in the traces; frequently it quits on the trail and has to be carried on the sledge to the next stopping post. In advanced stages, cachexia and marked emaciation are prominent (Fig. 1). When turned loose, the animal seeks seclusion and sometimes becomes lost in the bush. Often it wades into water and stands there in a stupor for a protracted period. Icterus was seen in one case, but it does not appear to be an outstanding feature of the disease. Prior to this investigation, the owner had noted nervous symptoms, simulating those seen in encephalitis, in a few of the diseased dogs.

Symptomatic anemia was very pronounced in all the cases coming under observation. Ascites, which appears to be a character of liver fluke disease in cats, was not evident in the cases under review.

Before death, the patient is so exhausted that it cannot rise without assistance, although the eyes may appear fairly bright and intelligent-looking. When raised to its feet, the animal stands as though propped on four sticks instead of articulating limbs (Fig. 1). The patient may remain in this condition for four or five days before death which is signallized by deep coma.

Some of the cases were complicated by a fairly heavy infestation with immature individuals of a species of *Diphyllobothrium*, probably *cordatum*, and it is difficult to say which part of the foregoing syndrome was referable to their presence.

Etiology

Description of the Parasite

The causative organism is a Trematode of the family Ophisthorchiidae Braun and the genus *Parametorchis* Skrjabin.

Four species of this genus have been previously recorded, namely:—*complexus*, originally described by Stiles and Hassall (4) from cats in New York, Maryland and District of Columbia, and more recently described by Essex and Bollman (1) from a cat at Rochester, Minnesota; *noveboracensis*, described by Hung (2) from a cat at Ithaca, New York: *intermedius*, described

by Price (3) from a specimen of *Vulpes fulva* in Wisconsin; and *canadensis*, described also by Price from a specimen of *Putorius vison* from Kirkfield, Ontario.

The present material shows a combination of characters differing from those of the four recorded species and in the writers' opinion it represents a hitherto undescribed species for which the name *manitobensis* is suggested.

From several hundred available specimens, 20 uninjured individuals were selected at random upon which to base the following specific description. The external dimensions are based upon 10 specimens preserved in 5% formalin; the internal dimensions are based upon the remaining 10 specimens, lightly stained with Grenachers borax carmine, cleared in beechwood creosote and mounted in Canada balsam.

Parametorchis manitobensis n.sp.

Specific diagnosis.—Parametorchis: body typically pyriform, although many individuals are linguiform, tapering cephalad, rounded caudad, translucent, minutely spinose; 5.5–6.6 mm. long by 1.7–2.6 mm. wide, greatest width between the testes or at level of posterior testis; oral sucker terminal, weakly muscular, wider than long, 25–40 μ long by 33–42 μ wide; acetabulum conspicuous, situated on low papilla, one-quarter to one-third of the body length from the oral sucker, weakly muscular, circular, 32–40 μ in diameter.

Prepharynx absent; pharynx strongly muscular, longer than wide, 25–35 μ long by 22–30 μ wide; esophagus very short; intestinal caeca sinuous, 18–25 μ wide, ending 20–25 μ from posterior body edge.

Genital pore median, at junction of first and second fifths of body length; ovary mediolateral, at middle of body length, oval to pyriform, long axis oblique and 60–80 μ in length; vitellaria in two lateral bands, extending cephalad from level of ovary to level of point midway between acetabulum and caecal fork, not united in front of uterus; uterus wholly in anterior body half, post-acetabular portion with transverse coils, pre-acetabular portion rosettiform; eggs 26–15 μ in dimensions; testes arranged tandem, wholly in posterior body half, markedly lobulate; anterior testis with three to five lobes, 50–70 μ long by 80–90 μ wide; posterior testis with five to nine lobes, 60–80 μ long by 65–85 μ wide.

Excretory stem sigmoid, between testes, nearly or as wide as caeca.

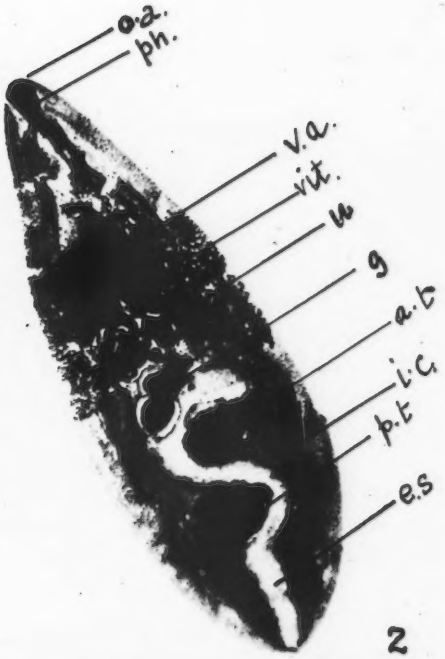
Host. Canis familiaris.

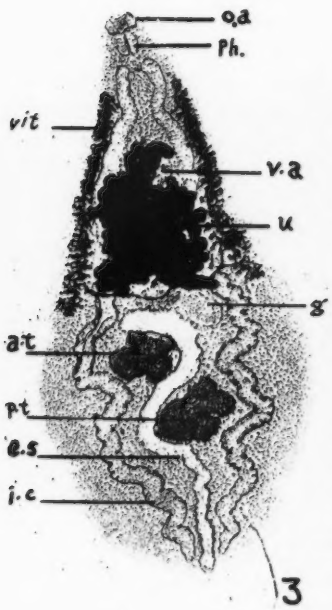
Location. Hepatic ducts.

Distribution. The Pas district, northern Manitoba.

Location of type of material. U.S.N. Museum.

From *complexus* this species is distinguishable by the absence of any preuterine commissure of the vitellaria; from *intermedius*, it is distinguished by its larger size, by the partly rosettiform uterus, by the wide excretory stem; from *noveboracensis* and *canadensis*, it is distinguished by the lobulated testes and the sinuous intestinal caeca (Figs. 2 and 3).





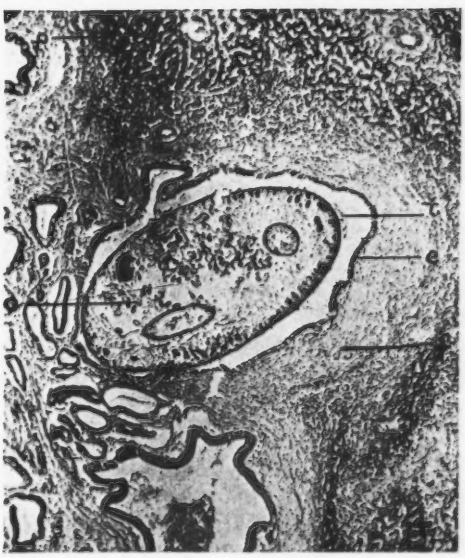


FIG. 1. *An advanced case of liver fluke disease.* FIG. 2. *Parametorchis manitobensis* × *16; o.a., oral sucker; p.h., pharynx; v.a., ventral acetabulum; u., uterus; vit., vitellaria; g., ovary; a.t., anterior testis; p.t., posterior testis; e.s., excretory stem; i.c., intestinal caecum. (Photographed without filter.)* FIG. 3. *As in Fig. 2; photographed through Wratten M (G-filter).* FIG. 4. *Section of liver* × *40; t., trematode in situ; o., ova of trematode; e., epithelium of bile duct; f., fibrosed wall of bile duct; p., cells of hepatic parenchyma.*

The following key to the known species of *Parametorchis* illustrates these differences:—

a_1. Testes markedly lobulate; intestinal caeca sinuous.

b_1. vitellaria uniting in front of uterus; body 5–7 mm. long..... *complexus.*

b_2. vitellaria not uniting in front of uterus;

c_1. uterus rosettiform, excretory stem wide, body 5–7 mm. long..... *manitobensis.*

c_2. uterus in transverse coils; excretory stem narrow; body 3–3.5 mm. long..... *intermedius.*

a_2. Testes smooth or only slightly lobulate; intestinal caeca straight.

d_1. uterus rosettiform; testes almost round; body length 6–6.3 mm..... *noveboracensis.*

d_2. uterus transversely coiled; testes oval or slightly lobed; body length 1.7–2.0 mm..... *canadensis.*

It may be noted that no opisthorchiid trematode has previously been recorded from the dog in North America, but *Amphimerus pseudofelineus* (Ward) was originally described from *Canis latrans* in Nebraska.

Pathology (Gross)

The outstanding pathological change is a hypertrophic cirrhosis of the liver, which is increased to two or three times the normal size and weight. In the field the writers were not equipped to weigh the specimens, but the body weight of the dog examined by Morrow was 56 lb. (25.40 kgm.) and the liver weighed 3 lb. 14 oz. (1.75 kgm.).

The liver is hard to the touch; its capsule yellowish in color, and its surface exhibits a varying number of hard, yellowish and circular elevations. The edges of the liver are markedly rounded.

When the organ is incised, a brownish green, fluke laden material oozes from greatly dilated bile ducts, some of which are as large in diameter as an ordinary lead pencil. In one of the fresh specimens the ducts were completely occluded by fluke laden exudate which appeared in motion when viewed through a binocular giving a magnification of eight diameters.

The wall of the gall bladder is thickened, and the bladder is engorged with dirty, greenish inspissated bile which teems with flukes and their ova.

Pathology (Microscopic)

Because of the enormous number of parasites in the specimens, no difficulty was experienced in obtaining microscope sections of liver showing the fluke, with its ova, *in situ* (Fig. 4).

In the few cases studied, the cells of the hepatic parenchyma were essentially normal, but demonstrable changes were evident in the bile ducts and interlobular connective tissue. The former had thickened fibrosed walls, sur-

rounded by areas of less-dense connective tissue. The interlobular connective tissue appeared to have undergone a simple hypertrophy. There was little or no evidence of congestion.

Transmission

Undoubtedly the direct vehicle of infection is raw lake fish. The severely affected dogs at Cormorant Lake were fed during the spring and summer months entirely on suckers (*Catostomus commersonii*), but the dogs from The Pas received in addition white fish (*Coregonus clupeaformis*), pickerel (*Lucioperca vitreum*), and lake trout (*Cristivomer namaycush*). The difference in diet may explain the difference in degree of infestation noted in these separate groups of dogs.

Since circumstantial evidence pointed to the sucker as the chief host of the metacercariae, attention was first directed to this fish. During the present winter 60 suckers were obtained from Cormorant Lake, but the writers have been unable to demonstrate the presence of the intermediate form in any of them. While extending the preliminary survey to include other kinds of lake fish, the writers are keeping in mind the possibility that suckers serve only as seasonal hosts of the metacercariae. Such seasonal occurrence would break the continuity of fluke infection in dogs, and give some time for establishing the relative resistance which seemingly exists against the disease.

Prophylaxis

Two groups of trappers' dogs, kept in runways within a few hundred yards from the affected animals at Cormorant Lake, were clinically free from disorder. One of these groups was fed on cooked fish, the other group exclusively on boiled cereals. The trapper who boiled the fish before feeding it to the dogs informed the writers that he and other dog breeders in the vicinity had experienced much difficulty in raising dogs prior to the adoption of this practice. He conveyed the idea that raw fish was falling into disrepute as a diet for sledge dogs, although the true cause was not suspected.

Acknowledgments

The writers' thanks are due to Mr. A. G. Cunningham, Director, Manitoba Game and Fisheries Branch, for permission to publish this report; to Dean Alfred Savage, University of Manitoba, for advice during the preparation of this report; to Mr. J. M. Isa for preparing some excellent tissue sections.

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OBSERVATIONS ON THE VARIABILITY OF *POLYSPORA LINI* LAFFERTY[1]

By A. W. Henry[2]

Abstract

Polyspora lini, the fungus which causes the browning and stem-break disease of flax, was isolated from flax material obtained from Ireland, Sweden, the United States and Canada. Cultural differences in pigmentation and growth characters were exhibited between different strains when grown on potato dextrose, corn meal and prune agars.

Saltants arose in the form of sectors from most of the parent cultures. The parent strain from Ireland was particularly prolific in the production of saltants. New strains differing from parental strains and others in cultural characters originated in this way. Many of these in turn produced sectors. Monosporous strains appeared to produce saltations as readily as polysporous strains.

Most of the original isolations yielded a special form of the fungus, characterized by the production on all media tested of firm, tough colonies consisting of abundant densely packed mycelia and a relatively sparse development of spores. On the same media the usual form of the fungus develops soft, loose, profusely sporulating colonies. Strains of the new form of the fungus have arisen as saltants either as sectors or as islands in colonies of the ordinary form of the fungus. They have thus far failed to revert to the parental type even after long culture on artificial media or on passage through the host plant.

Preliminary pathogenicity tests indicate that the same variety of flax may differ in its reaction to different strains of *Polyspora lini*, so that strains of this fungus may differ both in cultural characters and in pathogenicity.

Introduction

Cultures of *Polyspora lini* Lafferty, the fungus causing the browning and stem-break disease of flax have been under the observation of the writer for a period of 10 years. These cultures have been derived from diseased flax obtained from widely separated parts of the world and hence should be suitable for the detection of any variability which may exist in the fungus.

Previous Work

Relatively little has appeared in the literature concerning *Polyspora lini* since its original description by Lafferty (2) in 1921. His paper consequently still provides the principal information which we have on the morphology and cultural characteristics of the fungus and also on the disease which it causes. In respect to the constancy of its cultural characters he concludes that "irrespective of its source of origin, the fungus behaved similarly on the various media and showed no morphological or cultural differentiation into strains or races."

Sources and Isolation of the Fungus

Polyspora lini was first isolated by the writer in 1923 from diseased flax stems collected at Saskatoon in that year. It was again isolated in 1924 from

[1] *Manuscript received January 24, 1934.*
Contribution from the Department of Field Crops, University of Alberta, Edmonton, Alberta.

[2] *Associate Professor of Plant Pathology, Department of Field Crops, University of Alberta.*

flax leaves collected at Saskatoon in 1920 (1). In 1925 it was isolated from diseased flax stems obtained from the United States, Sweden and Ireland. The material from Ireland was of special value for comparative purposes as it was kindly supplied by Mr. H. A. Lafferty, the author of the original description of the fungus. Later in 1930 isolations were made from collections obtained from two points in the province of Alberta, Canada.* The sources of the various cultures and the collectors of the diseased material are indicated in Table I. The pathogenicity of most of the above collections has been proved.

TABLE I

SOURCES OF CULTURES OF *Polyspora lini* UNDER OBSERVATION

Culture	Country	Town	Part of flax plant cultured	Collector
A	Canada	Saskatoon, Sask.	Leaves	Henry
B	Sweden	Svalof	Stems	Sylven
C	Ireland	Dublin†	Stems	Lafferty
D	United States	East Lansing, Mich.	Stems	Robinson
E	United States	St. Paul, Minn.	Stems	Henry
F	Canada	Edmonton, Alberta	Stems	Henry
G	Canada	Provost, Alberta	Seed	Fullerton

†*Received from Dublin.*

The fungus was readily isolated after surface sterilizing bits of diseased tissue with mercuric chloride (1–1000) and plating them on potato dextrose agar. Profusely sporulating colonies typical of *Polyspora lini* commonly arose in a few days from such tissues. These usually were light in color at first, Plate II, Fig. 2, but soon turned dark owing to the production of a black pigment, Plate II, Fig. 3. In the case of the Swedish material, cultures were obtained by placing the diseased stems in a moist chamber to induce sporulation, suspending the spores in tubes of potato dextrose agar and sowing plates with the spore suspensions. Monosporous cultures were later made from most of the original isolations and from many of the variants which arose.

Media Employed

Since the fungus grew well on freshly prepared potato dextrose agar this medium was used for ordinary cultural work and for the preservation of the different isolations. In addition three commercial agars, namely Bacto potato dextrose agar (pH 5.5), Bacto corn meal agar (pH 6.1) and Bacto prune agar (pH 5.8) were used for comparative studies. As indicated below, these media proved effective in bringing out cultural differences between different isolations of the fungus. Had the same media been employed by Lafferty he undoubtedly would have obtained similar results. In his investi-

The assistance of two graduate students, Mr. E. G. Sharvelle and Mr. J. B. Skaptason, in isolating and photographing cultures of these Canadian collections is gratefully acknowledged.

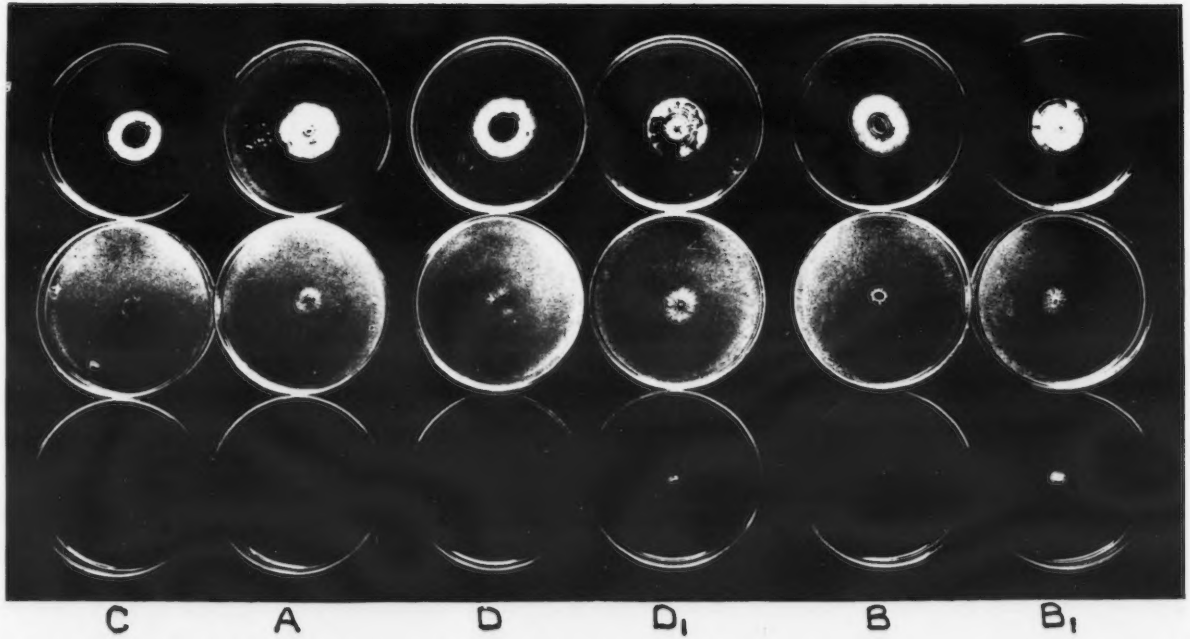

PLATE I

Comparison of cultural characters of six strains of Polyspora lini on three kinds of agar; namely, potato dextrose (left), corn meal (centre) and prune (right) after 11 days at room temperature. Each row of three plates represents a separate strain. These strains were derived from the following sources: C, from Dublin, Ireland; A, from Saskatoon, Saskatchewan, Canada; D, from East Lansing, Michigan, United States; D_1, from a sector of D; B, from Svalof, Sweden; and B_1, from a sector of B.

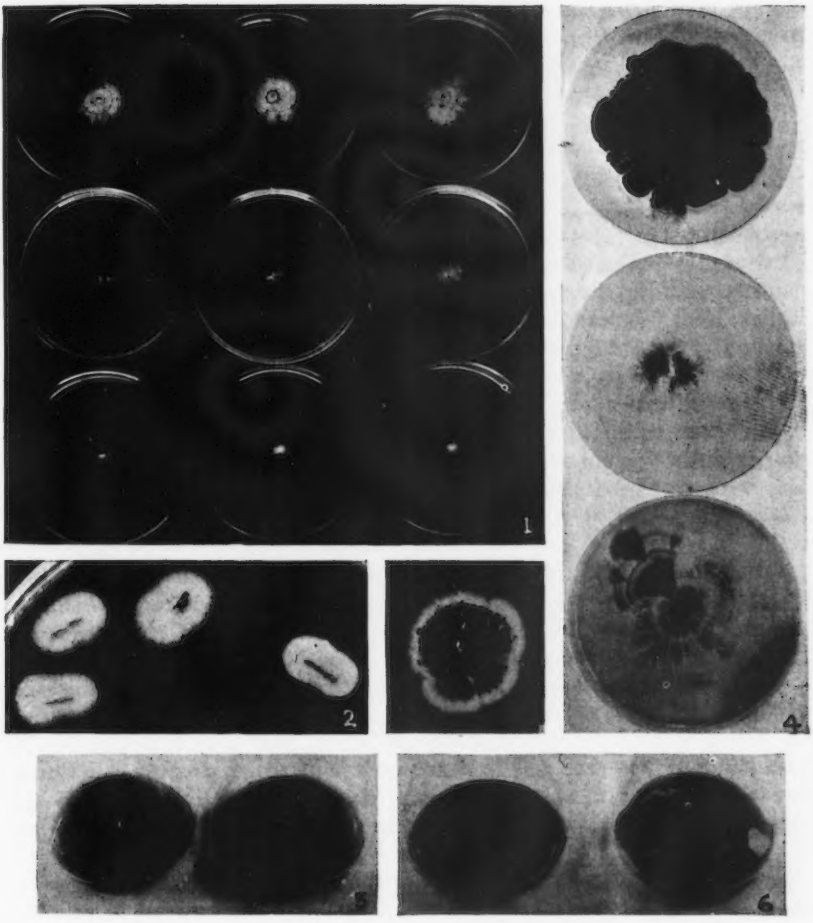

Fig. 1. *Photograph showing the uniformity of cultural characters of the monosporous strain B_1 of Polyspora lini, when grown on the same kind of agar. In the three plates at the top is shown the growth of the fungus on potato dextrose agar, in the three in the centre on corn meal agar, and in the three at the bottom on prune agar.* FIG. 2. *Young colonies of Polyspora lini on potato dextrose agar developing from pieces of diseased flax stem obtained from Ireland. These colonies had not as yet developed the characteristic black pigment.* FIG. 3. *Colony of Polyspora lini of polysporous origin obtained from flax from Sweden, showing sectors and the characteristic black pigment in the older parent part of the culture.* FIG. 4. *Photograph showing sectoring of monosporous strain C (from Ireland) of Polyspora lini on three different media; potato dextrose agar (top), corn meal agar (middle) and prune agar (bottom).* FIG. 5. *Cultures of two Canadian strains of Polyspora lini from Edmonton (left) and Provost (right) on potato dextrose agar, showing "islands" of the new sparsely sporulating form of the fungus.* FIG. 6. *Two isolates of the firm, sparsely sporulating form of the fungus each derived from an island similar to those illustrated in Fig. 5. That on the left was obtained from the Edmonton strain, and that on the right from the Provost strain. The marked differences in cultural characters from those of the parents are evident on comparison of Figs. 5 and 6.*

gations the fungus was grown on ground Quaker oat agar, beef extract agar, cooked flax extract agar and on beef extract, extract of Quaker oats, gelatin and cooked green flax stems. As already noted, the fungus behaved similarly on these media. It developed its mycelium comparatively sparsely and slowly in all cases, but always produced its conidia in great abundance.

Nature and Origin of Variability

In comparing the colony characters of cultures of *Polyspora lini* from different sources it was noticed that, while the majority resembled one another rather closely, there were some that differed quite widely from the rest. This is shown in Plate I by monosporous strains of the fungus from Ireland *C*, Canada *A*, the United States *D*, and Sweden *B*, growing on three different kinds of agar under similar conditions at room temperature. It will be noted that the strains from Ireland *C*, and the United States *D*, resemble each other closely on each of the three different media, whereas those from Sweden *B* and from Canada *A* differ considerably from *C* and *D* and also from each other. The most conspicuous difference is in the amount and distribution of the black pigment in the colonies. The Canadian strain *A* on potato dextrose and on corn meal agar remained relatively free from this pigment up to the time the photographs were taken, while the Swedish strain *B* produced an intermediate amount.

All the original strains *A* to *G* tended to produce sectors under certain conditions regardless of whether the parent cultures were derived from single spores or not. The strain from Ireland *C* was particularly prolific in the production of sectors, as is shown in Plate II, Fig. 4. Sub-cultures, both mass and monosporous, derived from these sectors commonly differed from their parents in pigmentation, habit of growth and other characteristics. In Plate I monosporous strains D_1 and B_1 obtained from sectors of *D* and *B* are compared with their parents on three kinds of agar. It will be noted that each differs from its parent on all three media. On prune agar B_1 shows particularly striking differences in habit of growth from its parent and also from all the other strains illustrated in Plate I.

Large numbers of saltants arising in the form of sectors have been observed, but only relatively few have been isolated. Saltation appears, however, to be the principal method of origin of new strains. In general, such strains exhibit as great a degree of constancy as their parents, producing a relatively uniform type of colony on a given medium. This is shown in Plate II, Fig. 1, in which B_1, a monosporous strain derived from a sector of *B*, is shown growing on triplicate plates of each of three different kinds of agar. The colonies as will be noted are quite uniform on the same kind of agar. However, on potato dextrose agar a tendency toward further variation is shown in the formation of sectors from which further strains might be derived.

Occurrence of Firm Sparsely Sporulating Strains

The usual type of colony of *Polyspora lini* described by Lafferty and observed by the writer is a flat, soft, profusely sporulating one, with a com-

paratively poorly developed mycelium. Such colonies are greasy in appearance and turn black with age. It was of interest, therefore, to encounter during the course of these studies a form of the fungus quite different from that just described. This contrasts with the ordinary form in producing colonies with relatively few spores but with vigorous well developed mycelia. The colonies may be described as firm, tough or leathery and are often more or less raised and furrowed. They are not greasy in appearance and vary in color from light to dark gray. Spores are produced, but in greatly reduced numbers compared with the usual form of the fungus. In fact the name *Polyspora* hardly seems appropriate for the new form. It would seem that the ability to produce this form is inherent in *Polyspora lini* generally, since in these studies it has been obtained from normal isolates of the fungus obtained from flax collections made in Ireland, the United States and Canada.

The new form of the fungus so far has shown greater constancy than the normal form on various media. Up to the present it has not reverted to the normal parental type. Some strains have retained their characteristics in culture for seven years. Even on passage through the host plant it has retained its characteristics. It has not as yet been derived directly from the host plant except following artificial inoculation with the same form of the fungus.

The strains of the new form so far observed have arisen from normal colonies only and in either of two ways: 1, in the form of sectors; and 2, in the form of islands (Plate II, Fig. 5). There has been no consistent difference observed between strains derived in the first way from those derived in the second way. In fact the origin may be the same in both cases, the only difference being in habit of growth afterwards. Though the new form of the fungus exhibits greater constancy than the normal form, different strains are not all alike on cultural media. There are differences in color of colony and perhaps in other characters between different strains of the new form.

Variation in Pathogenicity

Artificial inoculation studies have indicated that some varieties of cultivated flax, *Linum usitatissimum*, are more susceptible to *Polyspora lini* than others. The variety Bombay C.I. 42 and certain other Indian selections have proved among the most susceptible tested, whereas Winona, Ottawa 770B and others have shown considerable resistance. However, different strains of the fungus do not necessarily produce the same reaction on the same variety of flax. Thus one of the Indian selections, Type 12, developed distinct lesions when inoculated with strain *A* but none when inoculated with *C*. Bombay also developed distinct stem lesions when inoculated with strain *A* but none when inoculated with a hard (new type) saltant of *A*. This does not necessarily mean that all strains of the new type are of reduced pathogenicity compared with their parents. Further work will be necessary before this can be decided. In the case of saltants of the normal type evidence has been obtained that some show a reduction in pathogenicity while others exceed

their parents in this respect. The work done thus far on the host relations of the fungus then, while only of a preliminary nature, indicates that varieties of cultivated flax differ in their reaction to *Polyspora lini*, and that this fungus exhibits specialization in pathogenicity as well as in cultural characters.

Acknowledgments

The writer acknowledges with thanks the provision of facilities for part of these studies in other laboratories by the following: Dr. E. C. Stakman, University of Minnesota; Mr. F. T. Brooks, University of Cambridge; and Dr. Et. Foëx, Station Centrale de Pathologie Vegetale, Paris.

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EFFECT OF FROST ON WHEAT AT PROGRESSIVE STAGES OF MATURITY

I. PHYSICAL CHARACTERISTICS OF THE KERNELS[1]

By R. Newton[2] and A. G. McCalla[3]

Abstract

Samples of freshly cut wheat were exposed in freezing chambers, then stook-cured, threshed, and compared with unfrozen check samples, in the seasons 1929, 1930 and 1932, at Edmonton, Alberta. The check samples attained approximately maximum grade and weight per bushel when cut at a stage of maturity represented by a dry matter content ranging in different varieties and seasons from 50 to 60%. The weight per 1000 kernels of Marquis wheat, determined in 1930 only, reached a maximum at the same stage as weight per bushel. Susceptibility to superficial injury by frost continued generally over nearly the whole maturation period. Even 4 degrees of frost (28° F.) frequently caused a cut in grade, while 8, 10 and 14 degrees caused more substantial degradation, often accompanied by some reduction in weight per bushel. Marquis wheat is apparently more susceptible than Garnet, Reward and Red Bobs, to degradation by frost during the maturation period. Grade, being based on external appearance, is more sensitive to frost than are chemical composition and baking quality. Classification of the kernels of Marquis, in 1930, into vitreous, starchy, immature, green, bran frosted and heavily frosted, confirmed the earlier conclusion (2) that only the sound class (vitreous + starchy) has enough relation to grade to be useful as a grading factor. The germination of Garnet was improved by moderate freezing.

Introduction

Every year a certain proportion of western Canada's wheat crop is frozen before reaching maturity, and the effect of such freezing on the physical characteristics, chemical composition, and milling and baking quality of the grain is of great practical importance in relation to the problem of equitable grading and merchandising. In the fall of 1928 an unusually large proportion, in fact over half, of the crop was damaged by frost. The grades were low, the yields reduced in some cases, and the financial loss to the farmers exceedingly heavy. In view of this serious situation, the Associate Committee on Grain Research of the National Research Council undertook a study of various aspects of the problem.

The problem was attacked in two ways. First, a survey was made of the crop of 1928, the milling and baking quality of a large number of commercial samples being studied in relation to the grades given them as a result of frost injury. A report on this has been published by Geddes, Malloch and Larmour (2), and further surveys have been carried on annually, the results of which will be published in due course.

[1] *Manuscript received January 30, 1934.*

Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. Acknowledgment is made to the officers of the Western Grain Inspection Division, who graded all the samples, and of the laboratory of the Dominion Seed Branch at Calgary, who carried out the germination tests. Issued as Paper No. 49 of the Associate Committee on Grain Research.

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[3] *Research Assistant, Associate Committee on Grain Research, University of Alberta, Edmonton.*

The second method of attack was to produce artificially frosted grain of known history under controlled conditions. A freezing plant was installed at the University of Alberta, with which there was produced in 1929, 1930 and 1932 three series of samples subjected to graded frost exposures at progressive stages of maturity. These samples have provided the material for studies of physical characteristics, chemical composition, milling and baking quality, and seed value. This series of papers deals with the results obtained under the first three headings. The study of seed value has been carried out in co-operation with the Calgary laboratory of the Dominion Seed Branch, and will be reported later.

The work as a whole is adding substantially to the existing body of knowledge in regard to the properties and value of frosted wheat, and it is hoped that it may be of practical assistance in the consideration of grading problems.

Production of Material

In 1929, four varieties of wheat—Garnet, Reward, Marquis and Renfrew—were grown on clean fallow land (near Edmonton), and collections were made at progressive stages of maturity of the wheat, artificially frozen samples being produced from each. Cutting was started when the wheat kernels contained about 40% dry matter, it being judged, in the absence of experience on the point, that this was probably the earliest stage from which a milling sample could be secured. Sampling was continued until the wheat was ripe. The plots were long and narrow, and each cutting consisted of one swath the entire length of the plot.

About 45 sheaves of each of two varieties were cut as early in the morning as conditions would allow (usually between 8 and 9 o'clock). These were labelled in rotation as they fell from the binder, *A*, *B*, and Check, and directly afterwards brought by motor truck to the freezing plant. This plant had two chambers, which made it possible to freeze two lots at different temperatures each day. Each chamber contained two racks of chicken-wire shelves 6 in. apart, with a capacity of 10 sheaves of wheat cut open and spread out not more than 2 in. deep. The *A* and *B* sheaves were spread on the shelves in the inner and outer chambers respectively, and the ice machine started. The air in each chamber was circulated by means of a Sirocco fan connected with flues opening at various levels. An attempt was made to have the temperature curves simulate those of a natural frost. The rooms were cooled to minimum temperatures ranging from 30° to 18° F., these minima being maintained for one to two hours, after which the machine was shut off, and the doors opened.

Since a crew of two men was doing the sampling and freezing, it was necessary for them to remove the grain from the chambers in the evening, in order that the chambers might be free to receive the fresh samples they brought in the following morning. Thus the sheaves had to be retied and removed shortly after the doors were opened, a procedure that involved more rapid

warming up than would probably occur in nature. This was overcome in subsequent seasons by increasing the number of workers.

The *A* and *B* sheaves were stooked outside with the unfrozen check sheaves, and allowed to cure normally, except that all were capped with canvas covers at night and during rain, to prevent weathering. As soon as the grain was sufficiently dry, it was threshed. The samples were cleaned with special precautions to avoid the loss of small, frost-injured kernels, and were used for analytical and milling and baking studies.

In 1930, effort was concentrated on producing a homogeneous series of samples as long and complete as possible, for elaborate investigation. Only one variety, Marquis, was used. This was grown under conditions similar to those of the previous year, and sampled in much the same way, but beginning at an earlier stage, when the kernels contained about 30% dry matter. Moreover, profiting by the previous season's experience, when the frost exposures caused less damage than had been expected, the treatment in this respect was made more rigorous. With minor variations, the chambers were cooled to 28° and 24° F., and to 22° and 18° F., respectively, on alternate days, and the minimum temperature was maintained for four hours. The machine was shut off at the end of this period, but the wheat was left in place and the chambers kept closed till the following morning. The temperature therefore rose gradually, more nearly approaching natural conditions. True, the length of exposure to the minimum temperatures exceeded that usually found in a night frost, but in nature each spike of wheat stands separately and would doubtless cool more rapidly than under the experimental conditions described, notwithstanding the care taken to provide a good circulation of air.

In 1932, with the limited object of comparing the effect of frost on the grade and weight per bushel of a few well known varieties, and of producing seed for the co-operative study with the Dominion Seed Branch, the following varieties were used: Garnet, Reward, Red Bobs, Marquis. Sampling was deferred until the grain was sufficiently developed to be of interest from the practical grading viewpoint. Since smaller samples were adequate, it was possible to accommodate all four varieties in each frost chamber in a given run. The chambers were cooled to 28° and 22° F., respectively, held at the minimum temperature for about four hours, and allowed to warm up gradually.

This paper deals with the physical characteristics of the frozen wheat samples so produced in 1929, 1930 and 1932, in comparison with the corresponding non-frozen check samples. The chemical composition and milling and baking quality of the 1929 and 1930 series will be dealt with in succeeding papers of the series.

Previous Work

While it is not intended to present here a review of the literature on the physical characteristics of frozen wheat kernels, reference will be made to a few papers bearing directly on the points to be dealt with in the following pages.

Johnson and Whitcomb (3) published a valuable summary of information accumulated by themselves and others over a period of 10 years at the Montana Experiment Station, on both physical and chemical properties, and milling and baking qualities, of normal and frosted Marquis wheat. They found that when the kernels contained not more than 44 to 46% moisture, a stage they described as "stiff dough" and corresponding to the attainment of practically full kernel and bushel weight, frost appeared to have no further effect on milling and baking quality. Moisture content did not, however, furnish a clearcut line above or below which frosted kernels were either present or absent. Quite mature wheat showed a high percentage of wrinkled or blistered kernels after exposure and, other things being equal, this percentage largely determined the grade of the wheat. Frosted wheat had a lower weight per bushel than non-frosted wheat of the same stage of maturity. This was not due to a change in true density, but was attributed to the looser packing of the blistered kernels. Exposure to weathering further decreased the weight, an effect ascribed to leaching.

Arny and Sun (1), in experiments at St. Paul, Minnesota, found that both the kernel weight and bushel weight of Marquis wheat increased until within four days of maturity, though the change within a week of maturity was not sufficient to affect the grade. The kernels in the terminal spikelets were in the hard dough stage seven days before maturity, but in the lower spikelets did not attain this stage until two days before maturity. In line with the foregoing, Shutt (4) found that Reward wheat at Beaverlodge, Alberta, in 1928, cut at two-day intervals beginning at the early dough stage, attained the grade of No. 1 Northern and maximum kernel weight at the sixth cutting, though it was not considered ripe for normal harvesting until about two cuttings later. Garnet wheat in the same season at Lacombe, Alberta, cut at similar intervals, improved until it reached No. 2 Northern at the soft to medium dough stage, when it also attained its maximum kernel weight. Successive "slight frosts" beginning at this point, reduced all succeeding grades to Nos. 4, 5, 6 and Feed.

Geddes, Malloch and Larmour (2), as already noted, published the first report on the program of investigations on the grading of frosted wheat, of which the present paper forms a part. Since commercial grading is done on the basis of physical characteristics, these authors directed their enquiry chiefly to the relation between the proportions of such physical types as sound, frost-damaged, and immature kernels in a sample and its milling and baking quality. One chemical test was included, namely, the protein content, and this proved the best single measure of baking quality within each grade, though it was not a reliable measure when comparisons were made between the grades, owing to differences in protein quality. As grading factors for frost-damaged wheat, the only physical characteristics which were sufficiently related to milling and baking quality to promise usefulness were the weight per measured bushel and either the percentage of total sound or of hard red vitreous kernels.

Series of 1929

The grade and weight per measured bushel in relation to the stage of maturity and exposure to frost of the 1929 samples are shown in Table I.

TABLE I

GRADE AND WEIGHT PER BUSHEL IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST. SERIES OF 1929

Date of cutting	Dry matter at cutting, %	Degrees of frost, F.		Grade			Weight per bush., lb.		
		A	*B*	*A*	*B*	Check	*A*	*B*	Check
Garnet									
Aug. 1	43.4	7	4	5	4	4	60	60	60
Aug. 3	45.5	5	3	3	3	3	63	63	62.5
Aug. 6	49.0	8	6	3	3	3	64	64	63.5
Aug. 8	49.5	5	3	2	2	2	65	64	64.5
Aug. 12	57.4	4	2	3	2	2	65	64.5	65.5
Aug. 14	59.6	8	6	3	2	2	64.5	65	65.5
Aug. 16	60.6	9.5	8	3	2	2	65.5	65	65.5
Aug. 21	65.0	14.0	9	4	4	2	65	65	65.5
Reward									
Aug. 1	41.5	7	4	6	6	6	59	59.5	58
Aug. 3	42.9	5	3	5	5	5	63.5	63.5	63
Aug. 6	49.0	8	6	5	5	4	64	64	64
Aug. 8	48.2	5	3	3	3	3	65	65	65
Aug. 12	52.6	4	2	3	3	3	66	66	66
Aug. 14	54.3	8	6	3	3	2	66.5	66.5	66
Aug. 16	56.6	9.5	8	4	3	2	66.5	66.5	66
Aug. 21	63.0	14	9	5	5	2	66	66.5	66.5
Marquis									
Aug. 15	46.8	4.5	3	4	4	3	63	63	62.5
Aug. 17	48.8	8	6	3	3	3	64	64	63
Aug. 20	53.7	9	7	4	3	2	65	65	65
Aug. 22	54.9	9.5	8	5	4	2	65	65	65.5
Aug. 23	56.7	9	7	4	4	2	65	65.5	65.5
Aug. 24	57.6	8	6	4	3	2	65.5	65.5	66
Aug. 26	58.8	13	11	5	4	2	65	65.5	66
Aug. 27	59.0	9	7	5	4	2	65.5	65.5	66
Aug. 29	66.6	7	5	3	3	2	66	66.5	66
Renfrew									
Aug. 15	40.5	4.5	3	4	5	4	57	57	57.5
Aug. 17	46.2	8	6	4	4	4	59.5	59.5	59
Aug. 20	50.1	9	7	3	3	3	63	63	62.5
Aug. 22	52.7	9.5	8	5	4	2	63.5	64.0	64
Aug. 23	54.4	9	7	4	4	2	63.5	63.5	64.5
Aug. 24	52.3	8	6	3	3	2	64.5	64.5	64.5
Aug. 26	57.7	13	11	4	4	1	64	64	65
Aug. 27	56.5	9	7	4	3	2	64.5	65	65
Aug. 29	60.5	7	5	2	2	2	65	65	64.5

The stage of maturity is indicated by the dry matter content of the kernels at the time of cutting, it being believed that this is a safer and more precise measure of this property than the descriptive terms ordinarily employed, such as early milk, milk, late milk, early dough, etc., which depend largely on personal judgment. Not only does practical observation support such a belief, but also the study of chemical development in relation to moisture content to be reported in the succeeding paper. In 1929, the varieties were cut in pairs on any given day, the sampling of Garnet and Reward covering the period August 1 to August 21, and that of Marquis and Renfrew the period August 15 to August 29. Thus within each of these pairs the dry matter content at successive samplings may be taken to indicate their relative rates of progress towards maturity during these periods. Such a general relation may of course be interfered with, especially in later stages, by absorption of moisture in rainy weather.

The grade has been found more sensitive to frost than are composition and baking value. Although in 1929 the frost exposures caused less damage than had been expected, the effect of the more severe exposures is clearly seen in the grades. Nor is it confined to the more immature samples but occurs also in the most mature ones, as witness the last cuttings of Garnet and Reward. While these, it is true, suffered particularly severe exposure, it should be noted that 9 degrees of frost caused as much degradation as did 14 degrees. All varieties were judged to be fully ripe, from the standpoint of readiness for binder harvesting, when the last samples were taken, yet there is no evidence in the grades that they had become less sensitive to frost injury up to that stage. Wrinkling and blistering of the bran, in the absence of more deep-seated effects, are sufficient to degrade wheat seriously.

In the check (unfrozen) samples, the grades improved as the varieties progressed towards maturity, until the dry matter content reached about 50 to 55%, after which little further improvement took place that season. In regard to the relative resistance of the varieties to degrading by frost exposure, it is difficult to make any comparison because of the variations in freezing temperature, and the point will be left for consideration in connection with the 1932 series.

The weight per measured bushel of the grain, taken after curing and threshing, reached a maximum at the same stage at which the grades of the check samples became relatively constant. The weight is of course a factor in determining the grade. There was very little difference in the weights of frozen and non-frozen samples. In a few cases, where the grade was materially reduced by the frost exposure, the frozen wheat was slightly lighter. This agrees with common experience in regard to frosted wheat, the explanation usually offered being that the wrinkling or blistering of the kernels prevents such close packing in the bushel measure.

While it is not intended in this paper to discuss the seed value of frosted wheat, the laboratory germination percentages of the samples are given in

Table II because they show an interesting difference in the behavior of Garnet as compared with the other varieties. The frost exposures were not severe enough to injure significantly the germination of any of the varieties. In the case of Garnet alone, the germination of every sample was improved by the freezing treatment, a result for which no explanation can be offered at present, though it suggests a field for enzyme investigations.

TABLE II

GERMINATION IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST. SERIES OF 1929

Degrees of frost, F.		Dry matter at cutting, %	Germination, %			Dry matter at cutting, %	Germination, %		
			A	*B*	Check		*A*	*B*	Check
A	*B*	Garnet				Reward			
7	4	43.4	92	95	80	41.5	88	84	84
5	3	45.5	94	95	91	42.9	90	87	92
8	6	49.0	98	95	87	49.0	94	91	95
5	3	49.5	97	94	89	48.2	94	90	99
4	2	57.4	99	97	83	52.6	99	99	95
8	6	59.6	96	92	90	54.3	99	94	97
9.5	8	60.6	98	96	86	56.6	94	95	89
14	9	65.0	93	95	84	63.0	91	90	95
		Marquis				Renfrew			
4.5	3	46.8	98	98	98	40.5	100	98	99
8	6	48.8	98	98	99	46.2	100	99	96
9	7	53.7	98	97	98	50.1	98	100	93
9.5	8	54.9	100	99	99	52.7	99	94	88
9	7	56.7	97	96	99	54.4	93	92	74
8	6	57.6	98	98	98	52.3	87	95	97
13	11	58.8	94	94	97	57.7	93	95	92
9	7	59.0	93	92	99	56.5	84	89	86
7	5	66.6	98	98	98	60.5	91	90	83

Series of 1930

One feature of the 1930 samples requires explanation. The north end of the rather long, narrow block of Marquis wheat, five acres in extent, grown for this experiment, was exposed to soil blowing in the spring which cut back the young plants, then 2 or 3 in. tall, delaying their development and causing uneven ripening. The wheat in the more protected south end was in flower before that in the north end had finished heading. The first 10 cuttings were made from the south end, and the remainder were made some from the north end and some from the south end, but the wheat from the two ends was never mixed. In the tables, the samples are listed in the order of their dates of cutting, with those from the north end indicated by an asterisk. The grade, weight per measured bushel and weight per 1000 kernels of the 1930 samples, as affected by the freezing temperatures indicated at progressive stages of maturity, are shown in Table III.

TABLE III

GRADE, WEIGHT PER BUSHEL AND WEIGHT PER 1000 KERNELS OF MARQUIS WHEAT IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST (°F.). SERIES OF 1930

Dry matter at cutting, %	Grade**			Weight per measured bushel, lb.			Weight per 1000 kernels gm. (13.5% moisture)		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	*F*	*F*	*F*	43.0	45.0	43.0	13.50	13.59	12.86
34.9	*F*	*F*	*F*	46.5	49.5	49.5	16.58	18.18	16.99
39.4	*F*	*F*	*F*	50.5	52.0	53.0	19.75	20.55	19.55
43.8	*F*	5	4	55.5	57.0	57.5	23.38	24.28	23.77
46.8	*F*	5	4	57.5	57.5	58.0	26.13	25.64	25.89
48.3*	*F*	6	6	56.5	55.0	56.5	23.89	26.46	26.29
50.5*	*F*	*F*	5	55.5	57.0	59.5	26.47	28.91	28.43
51.1*	*F*	—	5	57.5	—	60.0	26.92	—	29.56
51.3*	*F*	6	5	58.5	58.5	58.5	29.21	28.05	27.12
55.8*	*F*	*F*	4	61.5	61.5	62.5	31.53	32.13	32.70
56.8	6	4	3	62.0	63.5	64.5	35.34	35.94	35.29
57.3	6	4	1	63.0	64.0	65.0	37.68	36.01	36.43
58.1*	5	—	3	61.0	—	62.0	35.10	—	35.50
59.1*	6	—	4	62.0	—	64.0	36.11	—	35.55
65.5*	3	—	3	63.5	—	63.5	35.97	—	33.18
65.6	5	—	2	62.5	—	64.5	36.96	—	36.96
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	*F*	*F*	*F*	43.0	43.0	48.0	14.54	14.32	15.50
38.1	*F*	*F*	6	47.5	49.0	51.0	16.85	16.91	18.50
40.9	*F*	*F*	4	54.0	53.5	56.0	21.20	21.18	22.20
45.5	*F*	*F*	4	57.0	57.0	58.5	23.64	24.23	25.40
46.7	*F*	*F*	4	59.0	58.5	60.0	26.59	27.24	26.98
50.5*	*F*	*F*	5	59.0	56.0	58.0	27.76	24.93	26.34
51.1*	*F*	—	5	59.5	—	57.5	26.88	—	29.56
53.3*	*F*	*F*	5	61.0	60.0	59.5	29.50	28.19	28.70
54.9	*F*	*F*	4	62.5	62.0	61.5	34.40	33.08	32.63
57.4*	*F*	*F*	3	61.0	61.0	62.5	33.08	33.90	32.39
58.1*	6	—	3	61.5	—	62.0	34.89	—	35.50
58.5	5	5	2	62.0	62.5	65.0	36.60	36.38	36.00
59.1*	6	—	4	62.0	—	64.0	35.73	—	35.55
65.5*	5	—	3	63.0	—	63.5	36.85	—	33.18
65.6	5	—	2	62.0	—	64.5	35.66	—	36.95
69.4	—	—	1	—	—	65.0	—	—	36.31

* *Samples from north end of the field.* ** *F = Feed.*

The earlier stage at which sampling was begun, as compared with 1929, and the more severe freezing treatment are reflected in the grades. The check samples improved as before with the progressive development of the kernels, though the official grades do not differentiate between them until a dry matter content of about 40% at cutting is reached. Below that they are all grouped together as "Feed". The upper limit of grade improvement is reached at a dry matter content of about 57%, as compared with about 54% for Marquis in 1929. The samples from the north end graded lower than those from the south end harvested at corresponding dry matter contents, because of the lack of uniformity and the presence of green kernels in the former, arising out of the uneven development of the crop in that part of the field.

Where the check sample is graded "Feed", it is of course impossible to judge frost effects by the grade of the corresponding frosted samples. In all other cases but one, an exposure to even four degrees of frost caused a lowering in grade. Such a relatively light frost for the most part caused no injury to the grade in the 1929 series, but the duration of exposure at the minimum temperature in any given run that year was only one to two hours as compared with four hours in 1930. It must not be concluded, however, that a night temperature falling to 28° F. and remaining there even a short time would not under natural conditions injure the grades of immature wheat standing in the field with each head exposed to maximum radiation on all sides. Experimental conditions cannot duplicate natural conditions exactly, but have the advantage of producing a series of comparable samples of which the history is fully known. An exposure to 8, 10 or 14 degrees of frost reduced to "Feed" all samples but two up to a dry matter content of 57.4%, the point at which the check samples attained their maximum grade value. The exceptions were two of the uniformly maturing samples from the south end of the field, with 56.8 and 57.3% dry matter, which after an exposure to eight degrees of frost graded No. 6. This is not to say that exposures to 8, 10, or 14 degrees of frost were equal in their effects, but merely that all the more immature samples so treated were thrown into the lowest grade, and no further differentiation can be made on this basis. In the more mature samples some difference in effect can be seen, but it is still relatively slight, owing in part to the narrower range of effect produced at this more resistant stage. But with one exception even the most mature samples were cut two or three grades by exposure to either 8 or 14 degrees of frost.

The weight per measured bushel recorded in Table III was determined after the samples had been stored together in a cool room for about four months, at which time they all contained close to 14% moisture. In earlier work in the laboratories co-operating with the Associate Committee on Grain Research, the practice had been tried of determining the weight per bushel when the wheat had been tempered to 13% moisture in preparation for milling. It was soon found that the weights so determined were so much influenced by the previous history of the samples, particularly by their moisture content just before tempering, as to be unreliable. The practice now is to determine both weight and moisture content of samples as received in the laboratory, and if possible to handle all the samples in a given series in such a way that they may be kept at about the same moisture content and never dried excessively before weighing.

The weight per bushel of the check samples rose from 43 to 65 lb. during the experiment, reaching the latter maximum when the kernels contained 57 to 58% dry matter at the time of cutting. Again, as in 1929, the attainment of maximum weight coincided roughly with the attainment of maximum grade. Unlike the 1929 freezing treatment, which had little effect on weight, the more rigorous exposures of 1930 lowered the weight appreciably in most cases. The exceptions may perhaps be attributed to sampling errors.

The weight per 1000 kernels, corrected in Table III to a moisture content of 13.5%, followed very much the same course in relation to stage of development as did the weight per bushel, reaching a maximum at the same dry matter content, namely, 57 to 58%. There are no differences in either check or frosted samples after this point which may not reasonably be laid to sampling errors. Differences between the check and frozen immature samples are considered to involve a further factor, namely, the balance between translocation and respiration after the treated grain had been stooked for curing. Exposure to 10 and 14 degrees of frost is assumed to stop both these processes very largely, and the first four samples so treated are definitely lighter than their checks, apparently having lost more by the stoppage of translocation than they saved by the stoppage of respiration. The samples exposed to 4 and 8 degrees of frost are irregular in weight, but on the whole just as heavy as their checks, indicating that these exposures checked translocation and respiration only partially, if at all. More data on translocation and respiration in stooked grain are given in the next paper of this series.

Physical Classification of Kernels

A physical classification of the kernels in the 1930 samples, similar to that described in the report by Geddes, Malloch and Larmour (2) reviewed briefly in the introduction to this paper, was carried out by an experienced grader formerly on the staff of the Western Grain Inspection Division. Approximately 1000 kernels of each sample were classified, kernel by kernel, as vitreous, starchy, immature, green, bran frosted, or heavily frosted. All kernels showing blisters or "scalding" were classified as frozen. Those on which the blistering was carried down into the crease were grouped under *heavy frost*, all others under *bran frost*. *Green* kernels were very immature and shrunken, with the green color persisting. *Immature* kernels did not show external evidence of frost, but could not be classified as green or sound; they are commonly bronze or pinkish in color. Sound kernels were divided into *vitreous* and *starchy*, the latter class including any noticeably piebald kernels.

The results of this classification are given in Table IV. The proportions of the various classes, though first determined by number, are reported in percentages by weight, since this is the basis used in the official grade specifications.

This method of classification proved to be inapplicable to very immature wheat, since it is impossible to distinguish sharply between kernels which are shrunken merely from lack of development and those which are also frost injured. The percentage of so-called green kernels is in fact increased by more severe freezing, at the expense partly of the frozen class, because of the "fixing" of the chlorophyll brought about by the freezing treatment. This effect is very noticeable in the second sample, cut at 34% dry matter. The same difficulty occurs, though in less degree, with the more mature samples, in a number of which the apparent percentage of heavily frosted

TABLE IV

PHYSICAL CLASSIFICATION OF MARQUIS WHEAT KERNELS, BY WEIGHT, IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST. SERIES OF 1930

Dry matter at cutting, %	Degrees of frost, F.	Vitreous, %	Starchy, %	Total sound, %	Immature, %	Green, %	Bran frost, %	Heavy frost, %	Total frost, %	Grade**
31.2	8	0	0	0	82.1	2.9	0	14.9	—	*F*
	4	0	0	0	96.2	0.5	0	3.3	—	*F*
	None	0	0	0	98.9	0.5	0	0.5	—	*F*
34.0	13.5	0	0	0	53.4	27.0	0	19.6	—	*F*
	9	0	0	0	67.1	16.7	0	16.1	—	*F*
	None	0.6	0	0.6	97.0	0.9	0	1.5	—	*F*
34.9	8	0.8	0	0.8	81.9	4.2	0	13.0	—	*F*
	4	2.0	0	2.0	93.0	0.9	0	4.1	—	*F*
	None	1.8	0	1.8	97.6	0.6	0	0	—	*F*
38.1	14	0	0	0	61.8	4.6	1.3	32.3	—	*F*
	10	1.1	0	1.1	74.5	4.4	0.8	19.3	—	*F*
	None	6.0	0	6.0	93.3	0.7	0	0	—	6
39.4	8	0	0	0	62.3	4.4	0	33.2	—	*F*
	4	0	0	0	78.4	0.6	5.5	16.4	—	*F*
	None	1.8	0	1.8	97.8	0.3	0	0	—	*F*
40.9	14	0	0	0	55.9	9.3	2.6	32.2	—	*F*
	10	0.5	0	0.5	51.6	7.5	3.7	36.7	—	*F*
	None	8.2	1.2	9.4	90.0	0.6	0	0	—	4
43.8	8	2.4	0	2.4	50.1	6.2	4.9	36.3	—	*F*
	4	5.2	1.5	6.7	60.9	1.8	3.8	26.8	—	5
	None	10.3	2.2	12.5	85.8	1.8	0	0	—	4
45.5	14	0	0	0	47.3	17.3	7.5	27.9	48.8	*F*
	10	3.1	0	3.1	56.1	10.2	5.6	25.0	45.7	*F*
	None	48.8	0	48.8	50.9	0.3	0	0	—	4
46.8	8	5.3	0	5.3	44.9	10.0	0	39.8	43.4	*F*
	4	12.9	0	12.9	46.2	1.8	14.2	24.9	35.8	5
	None	48.7	0	48.7	49.2	2.1	0	0	—	4
46.7	14	1.1	0	1.1	58.4	8.0	4.5	27.9	43.1	*F*
	10	2.2	0	2.2	50.7	6.9	3.3	36.9	42.0	*F*
	None	42.6	1.6	44.2	54.0	1.8	0	0	—	4
48.3*	8	0	0	0	39.8	10.1	2.5	47.7	—	*F*
	4	2.0	6.0	8.0	56.1	1.4	8.2	13.8	—	6
	None	6.8	4.8	11.6	76.4	11.3	0.6	0	—	6
50.5*	14	0	0	0	47.1	14.4	5.1	33.4	78.7	*F*
	10	0	0	0	38.4	5.8	1.0	54.9	78.8	*F*
	None	72.1	6.6	78.7	17.9	3.4	0	0	—	5
50.5*	8	0	0	0	35.2	11.1	3.8	50.0	80.6	*F*
	4	0	0	0	19.3	5.8	10.4	64.4	80.5	*F*
	None	79.1	1.3	80.4	13.6	5.9	0	0	—	5
51.1*	14	0	0	0	36.0	11.6	3.8	48.6	80.5	*F*
	7	0	0	0	27.3	10.7	0.8	61.2	80.5	*F*
	None	79.7	0.8	80.5	15.4	4.1	0	0	—	5
51.3*	8	0	2.2	2.2	24.4	7.7	0	65.7	78.4	*F*
	4	14.6	0	14.6	27.3	10.9	22.6	24.5	65.9	6
	None	78.9	1.8	80.7	12.4	7.0	0	0	—	5

TABLE IV—*Concluded*

PHYSICAL CLASSIFICATION OF MARQUIS WHEAT KERNELS, BY WEIGHT, IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST. SERIES OF 1930

Dry matter at cutting, %	Degrees of frost, F.	Vitreous, %	Starchy, %	Total sound, %	Immature, %	Green, %	Bran frost, %	Heavy frost, %	Total frost, %	Grade**
53.3*	14	0	0	0	44.4	13.4	2.1	40.0	85.1	*F*
	10	0.6	0	0.6	40.6	12.7	0.8	45.3	84.6	*F*
	None	83.1	2.1	85.4	11.9	2.9	0	0	—	5
54.9	14	2.0	0	2.0	12.6	2.2	12.4	71.0	91.3	*F*
	10	0	0	0	5.1	3.4	5.7	85.7	93.0	*F*
	None	90.5	2.1	92.6	4.4	2.5	0	0.4	—	4
55.8*	8	1.4	0	1.4	16.4	7.0	6.9	68.3	91.8	*F*
	3	5.2	0	5.2	8.4	4.4	2.6	79.4	88.0	*F*
	None	91.4	1.5	92.9	5.9	0.9	0	0	—	4
56.8	8	7.4	0.3	7.7	11.3	7.7	10.2	63.0	90.6	6
	4	64.4	1.0	65.4	6.7	0.6	6.1	21.1	32.9	4
	None	96.4	1.5	97.9	1.5	0.1	0	0.4	—	3
57.3	8	6.6	1.4	8.0	1.4	0.1	5.5	85.0	91.0	6
	4	36.6	5.3	41.9	0.9	0.6	14.8	42.3	57.6	4
	None	92.2	6.8	99.0	1.0	0	0	0	—	1
57.4*	14	3.3	0	3.3	9.6	12.7	16.9	57.5	86.6	*F*
	10	3.0	0.6	3.6	17.4	9.6	15.5	53.9	86.3	*F*
	None	87.5	2.4	89.9	9.9	0.2	0	0	—	3
58.1*	14	12.6	0	12.6	14.8	5.1	7.5	59.9	78.3	6
	7.5	14.9	8.3	23.2	11.9	2.1	19.0	43.8	69.3	5
	None	66.1	8.5	74.6	8.6	0.4	16.4	0	—	3
58.5	14	9.9	1.9	11.8	1.5	0.4	4.3	82.0	86.3	5
	10	11.2	0	11.2	0.6	0.6	3.9	83.7	86.9	5
	None	95.3	2.3	97.6	1.9	0	0	0.5	—	2
59.1*	14	9.4	0	9.4	3.5	7.4	10.5	69.1	81.2	6
	8	16.9	0	16.9	2.3	4.3	12.1	64.4	73.8	6
	None	86.2	2.6	88.8	9.3	0	1.9	0	—	4
65.5*	14	24.6	0	24.6	2.2	5.7	16.1	51.4	71.5	5
	7	76.3	5.1	81.4	4.7	0.7	11.8	2.1	15.8	3
	None	92.0	3.1	95.1	3.9	0	1.0	0	—	3
65.6	14	10.9	0	10.9	1.1	2.9	9.4	75.7	86.7	5
	7	19.9	0.4	20.3	2.2	1.8	7.2	68.5	77.3	5
	None	95.3	2.3	97.6	2.4	0	0	0	—	2
69.4	None	96.7	2.6	99.3	0.6	0	0	0	—	1

* *Samples from north end of field.* ** *F = Feed.*

kernels bears an *inverse* relation to the severity of freezing exposure. Here an attempt was made to show the real effect of frost by inserting in Table IV the column headed "Total frost". This is calculated by adding together the percentages of immature, green, bran frost and heavy frost in the samples exposed to frost, and subtracting the sum of the immature and green in the

unfrozen check samples. The device was only partially successful since, with a few exceptions, the values for total frost were too constant to provide a good basis of differentiation.

Up to a dry matter content of 40% at cutting, nearly all the kernels of the unfrozen checks were included in the immature class. The traces of frost indicated in the first two and in a few later check samples are probably attributable either to imperfect cleaning of the thresher between samples or to error in judgment in classifying.

At least a few sound kernels were found in all check samples but the first one, though the percentages were negligible till the grain reached a dry matter content of 40% at cutting. After this they rose rapidly, nearly half the kernels being sound at 45 to 47% dry matter, although that corresponded to the milk stage. This shows the considerable development in the stook while drying. From the time the grain contained 50% dry matter at cutting, at least 80% of the kernels were sound in most samples. The percentage was somewhat influenced by the section of the field from which the samples were cut, those from the north end containing a higher percentage of green and immature kernels, owing to the delayed development and uneven ripening of the crop already described. In all samples, the sound kernels were mainly vitreous, the highest proportion of starchy kernels being 8.5%.

The lack of uniformity in the north end samples was such an important factor in grading as largely to obscure the relation between percentage of sound kernels and grade in the unfrozen checks. In the frozen samples, notwithstanding the more limited operation of the same factor, the relation between percentage of sound kernels and grade is fairly evident in the following brief census:

Feed grade—	18	samples	contained	no sound kernels
	16	"	"	0.5 to 5.3% sound
Grade No. 6—	7	"	"	7.7 to 16.9% "
" " 5—	8	"	"	6.7 to 24.6% "
" " 4—	2	"	"	41.9 and 65.4% "
" " 3—	1	"	"	81.4% "

(No frozen samples graded higher than No. 3 Northern.)

As already indicated, the study of Geddes, Malloch and Larmour (2) on commercial samples led to the conclusion that, of the various quantities derived from the physical classification of kernels, only the percentage of sound kernels (or of its best fraction, the vitreous kernels) promised usefulness as a grading factor. They were working with heterogeneous samples, and it seemed possible that with the basically homogeneous material of the 1930 artificially frozen series more precise results might be obtained. It is doubtful, however, that any of the classes shown in Table IV are worthy of consideration as an index of quality, other than the sound kernels.

The limitations of any grading system based wholly on the physical characteristics of the kernels appear plainly in the degradation of the most mature samples exposed to frost. The sample cut when it contained 65.6% dry

matter, a stage riper than necessary for normal harvesting with a binder, had over 75% of its kernels marked by exposure to seven degrees of frost, and was cut three grades, without any corresponding injury to quality which could be detected by chemical or baking tests.

Series of 1932

The effect of freezing at different stages of maturity on the grade and test weight of Garnet, Reward, Red Bobs and Marquis samples produced in 1932 is shown in Table V and Fig. 1. These varieties during the seven-year period

TABLE V

EFFECT OF FREEZING AT DIFFERENT STAGES OF MATURITY (AS SHOWN BY DRY MATTER CONTENT OF KERNELS AT TIME OF CUTTING) ON THE GRADE AND TEST WEIGHT. SERIES OF 1932

Date of cutting	Degrees of frost, F.	Garnet			Reward			Red Bobs			Marquis		
		Dry matter, %	Grade*	Wt. per bush., lb.	Dry matter, %	Grade*	Wt. per bush., lb.	Dry matter, %	Grade*	Wt. per bush., lb.	Dry matter, %	Grade*	Wt. per bush., lb.
Aug. 4	None	51.1	3	63.5	46.2	4	64	—	—	—	—	—	—
	10		*F*	62		*F*	62	—	—	—	—	—	—
Aug. 6	None	56.2	3	64	50.7	3	65.5	—	—	—	—	—	—
	4		3	64		3 *p*	65	—	—	—	—	—	—
	10		5	64		*F*	64.5	—	—	—	—	—	—
Aug. 9	None	70.8	2	63.5	57.6	2	66.5	—	—	—	—	—	—
	4		2	63.5		2 *g*	66	—	—	—	—	—	—
	10		3 *g*	64		5 *p*	65	—	—	—	—	—	—
Aug. 13	None	70.9	2	64	58.2	1 *H*	66.5	55.0	1 *H*	65	57.2	2	63.5
	4		2	64		3	66		1	64.5		4 *g*	63.5
	10		3	64		5 *p*	65		6	61		*F*	63
Aug. 15	None	78.5	1	64	62.7	1 *g*	66.5	55.4	1 *H*	65	60.5	1	64.5
	4		1	64.5		3	66		2	65		2	64
	10		2 *p*	64.5		4	65.5		6	62		*F*	63
Aug. 17	None	80.5	1	64	75.1	1 *H*	66	59.6	1 *g*	65	62.8	1	65
	4		1	64		1 *H*	66		2	65		2	65
	10		2	64		2	66		5 *g*	64		*F*	63
Aug. 19	None	84.8	1	64.5	78.5	1 *H*	66	62.4	1 *H*	65	76 5	1 *H*	65
	4		1	64.5		1 *H*	66		1 *H*	65		1 *H*	65
	10		(1)	64		1 *H*	66		3 *p*	65		6	64
Aug. 22	None	85.7	1	64.5	86.8	1 *H*	66	68.8	1 *H*	65	78.4	1 *H*	65.5
	4		1	64		1 *H*	66		1 *H*	65		1 *H*	65.5
	10		(1)	64		1 *H*	66		1	65		5	64
Aug. 24	None	—	—	—	—	—	—	74.4	1 *H*	65	81.9	1 *H*	65.5
	4	—	—	—	—	—	—		1 *H*	65		1 *H*	65
	10	—	—	—	—	—	—		1	65		2 *g*	65
Aug. 26	None	—	—	—	—	—	—	82.2	1 *H*	65	89.3	1 *H*	65
	4	—	—	—	—	—	—		1 *H*	65		1 *H*	65
	10	—	—	—	—	—	—		1 *H*	65		1 *p*	65

* *1 H = 1 Hard; F = Feed; g = good; p = poor.*

1926-32 at Edmonton had an average growing season of 101, 102, 104, and 107 days respectively. In the particular year of this experiment (1932) the periods were 94, 96, 98 and 99 days. Their relative earliness is reflected in the dry matter content of the kernels at any date of cutting shown in Table V, except that the positions of Red Bobs and Marquis are unexplainably reversed. The sensitivity of the two latter to frost damage on any given date, as indicated by the effect on the grades shown in the table, is however proportional to their recognized habits of maturation rather than to the moisture content of the kernels. This, it may be remarked in passing, is the only instance encountered throughout these investigations where dry matter content did not appear to be a reliable index of maturity.

An arbitrary factor interfered with the interpretation of the grades as first recorded, in that the grading regulations exclude Garnet from grades higher than No. 2 Northern because of varietal differences in quality. The Garnet samples were regraded with this limitation removed, but unfortunately the last two samples were missed on this occasion, and the grade No. 1 Northern indicated in brackets is a presumption based on the trend of preceding samples in comparison with the corresponding trend in other varieties.

Exposure to four degrees of frost, up to about 60% dry matter, cut some samples of Reward and Red Bobs and all samples of Marquis one or two grades. Only one sample of Garnet so exposed came within this dry matter category, and it was unaffected. Exposure to 10 degrees of frost cut the grades of Reward and Red Bobs up to a dry matter content of about 75%, of Garnet up to about 80%, and of Marquis over its entire dry matter range, which extended to 89.3%. This heavier frost differentiated Marquis from the other varieties, the former being apparently more sensitive to degradation by frost during the whole maturation period (Fig. 1). The differences between the other three varieties are probably not significant.

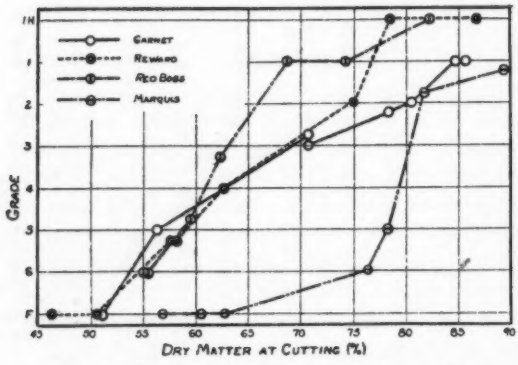

FIG. 1. *Grades of four wheat varieties exposed at progressive stages of maturity to 10 degrees of frost for four hours.*

The reduction in grade by exposure to four degrees of frost was accompanied either by no change or only a very slight reduction in weight per bushel. The more severe blistering caused by the 10-degree frost had in several, though not in all, cases an appreciable effect on weight per bushel. The earlier samples, as might be expected, were more reduced in weight than the later ones.

In the unfrozen checks the weight per bushel reached a maximum in samples containing 55 to 60% dry matter at cutting, a stage perhaps slightly later than in the corresponding varieties in 1929 and 1930. The grades of Reward and Red Bobs also reached their best in this dry matter range, but Garnet and Marquis improved one more grade at a later stage. This may be accounted for in Garnet by the removal in 1932 of the arbitrary factor in its grading already referred to, while in Marquis the change from No. 1 Northern to No. 1 Hard (a grade attained only in 1932) has but restricted significance, since in these high grades discrimination is quite rigid and differences are comparatively small.

There is on the whole a good agreement in the results of all three seasons with respect to the stage at which grade and weight per bushel of unfrozen wheat reached an approximately constant level. Susceptibility to degradation by exposure to frost continued in all cases for a considerable time thereafter.

The extent to which such superficial injury, unaccompanied by any changes in internal quality which can be detected by chemical or baking tests, affects the real value of the wheat, is a very difficult point to assess. When blistering is severe enough to reduce the weight per bushel, it is probably safe to assume that the milling yield of flour will also be reduced. There seems little doubt, however, that the heavy cut in grade resulting from superficial frost injury is often disproportionate to the real change in commercial value.

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AMIDE NITROGEN IN GERMINATING SEEDS[1]

By A. G. McCalla[2]

Abstract

Experiments with cereal seeds indicate that the amide groups of reserve seed proteins are more readily removed from the protein during germination than are the other structural units of the protein. No accumulation of amides was found in the non-protein nitrogen of seeds nor in seedlings.

Introduction

There is a considerable volume of literature dealing with the distribution of nitrogen fractions in germinating seeds, but little of it refers to the cereals. With regard to the amide fraction of the nitrogen, Kostychev (3) sums up the older work as follows: ". . . . it can be considered as established that only a small quantity of amides are formed directly by the hydrolysis of reserve proteins. Most of them are the result of synthesis at the expense of the other crystalline structural units of the protein molecule." Kostychev's review covered work by Schulze, Prianischnikov, Zaleski, Butkewitsch and others, all of whom studied principally legume seeds. In one experiment it was reported that 73% of the nitrogen from decomposed proteins was accounted for by the amide asparagine.

Kostychev's conclusion has not been entirely confirmed by more recent work, even when its application is limited to legumes. It is important to note that most of the results he considered were obtained in studies in which the materials analyzed had been previously dried at various temperatures. He cites the work of Schulze and his school as outstanding in the field, but Schulze and Castoro (6) dried most of their material at 60 to 70° C., under which conditions Chibnall (2) has shown that the asparagine content is greatly increased as a result of the activity of enzymes.

Sure and Tottingham (7) found that amides accumulated in the shoots of germinating peas, but in much smaller amounts than had been reported previously. More recently, Bonnet (1) studied lupin seeds and seedlings during germination and found that the decrease in protein nitrogen of seeds could be accounted for by increases in amide and amino nitrogen. The total increase in amino nitrogen, however, was greater than that in amide nitrogen.

None of these investigators determined the composition of the proteins remaining undecomposed at the time the other analyses were made. Chibnall

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(2) found that enzymatic decomposition of protein during low-temperature drying of leaves decreased the amount of protein but did not change the character of that portion remaining undecomposed.

Sasaki (5) fractionated the heat-coagulable protein of germinating soy bean seeds. Although he presents no data, he states that no intramolecular change of the protein was indicated from his results.

Detailed experiments with cereal seeds are scarce. Takahashi and Shirahama (8) germinated hulless barley and studied the nitrogen distribution. Unfortunately they also dried their material before analysis. They found a decrease in protein during germination, and since this decrease was particularly great with the alcohol-soluble protein, they prepared hordein from germinated and ungerminated seeds. They then fractionated the preparations, and their results indicated no significant change in composition during germination. It must be noted, however, that none was to be expected, as any marked change in composition would probably change the physical properties to such an extent that the altered portion would be lost during preparation.

A general survey of the literature leaves some doubt as to the validity of generalizing Kostychev's conclusion to cover all seeds. During the course of work on nitrogen metabolism of cereals, the writer obtained evidence that the amide groups of reserve proteins were more readily removed from the protein during decomposition than were the other nitrogen groups. It was thought that this relation might hold for decomposing seed proteins during the early stages of germination. If it did, it would offer an alternative explanation for the accumulation of non-protein amides noted by other workers in this field. It is believed that the results presented in this paper indicate a need for revising Kostychev's conclusion, at least in its application to cereals.

Methods

Wheat, barley, rye, corn and pea seeds were studied. The seeds were soaked overnight in tap water, then spread out on cheesecloth over water. Two samples of wheat (1*b* and 2*b*) were allowed to germinate until the seedlings just began to split the seed coat, and then were analyzed. All other samples were allowed to germinate three days, after which the seedlings were separated from the seeds, and the latter extracted and analyzed. The methods used for extraction and analysis have been fully described in another paper (4). In this experiment protein nitrogen, and amide, humin, and basic fractions of the protein were determined for the majority of samples. The non-protein nitrogen of two pairs of wheat samples (1*a* and *c*, 2*a* and *c*) were fractionated, and two lots of wheat seedlings were analyzed.

In separating the seedling from the seed it was impossible to get an exact separation, because parts of the seedlings were contained within the seed coat. In every case the procedure adopted was to cut the seedling from the seed, leaving with the seed all those parts inside the seed coat. Thus the total

protein decreases in germinated seed recorded in Table I are too low, because some of the protein nitrogen recorded as undecomposed protein had been regenerated in the part of the seedling left within the seed.

Results

The results of protein analyses are presented in Table I. The decrease in protein nitrogen during germination has been noted by all other workers in this field. The humin results are not presented because any differences occurring are less than the experimental error. Basic nitrogen expressed as percentages of the protein nitrogen undergoes a slight increase during germination. When expressed in terms of weight changes, although there were apparently some decreases and some increases, these were negligible as compared with the changes in amide nitrogen.

In marked contrast to the results for humin and basic nitrogen, those for the amide fraction of the protein show that the decrease in this fraction accounts for most of the decrease in protein nitrogen. Some of the results, in fact, indicate that there was more amide nitrogen split from the protein than there was protein decomposed. These results, obtained only with wheat, are probably explained by the incomplete separation of seedlings from the seeds as described under Methods. It is possible that some intramolecular change, by which some of the amide was converted to a non-amide form, took place within the reserve protein itself, but this does not seem likely. Despite the inaccuracies of the methods, however, there can be no doubt that the total amide nitrogen of the protein was greatly reduced during germination.

The results for non-protein nitrogen of seeds and for total nitrogen of seedlings are presented in Table II. They do not indicate any accumulation of amides at the expense of the other structural units of the reserve protein. In this experiment, the other nitrogen compounds must have increased at the expense of the amide split from the protein reserves. The total amide nitrogen present in the non-protein nitrogen of seeds and in seedlings accounts for only 15%, in the case of sample 1*c*, and 25%, in the case of sample 2*c*, of the amide removed from the seed proteins.

The results with peas indicate no removal of amide groups during germination. The metabolism of legumes is known to be quite different from that of cereals, and the conclusions drawn by workers who have confined their attention to these plants, cannot be refuted by the results of this experiment.

The results suggest, however, that it is necessary to modify the general conclusions which have been applied to all types of seed plants. The results obtained with wheat are definite, and those with all other cereals support the conclusion that the amide groups of the reserve proteins are more readily removed during the early stages of germination than are the other structural units.

TABLE I

NITROGEN DISTRIBUTION IN UNGERMINATED AND GERMINATED SEEDS

Sample No.	Seed	Condition	Total nitrogen, %	Protein nitrogen			Amide nitrogen of protein				Basic nitrogen of protein			
				% of total N	Mgm. per 1000 seeds	Decrease in germinating, mgm.	% of protein N	Mgm. per 1000 seeds	Decrease in germinating		% of protein N	Mgm. per 1000 seeds	Decrease in germinating	
									Mgm.	% of protein N decrease			Mgm.	% of protein N decrease
1a	Wheat	Ungerminated	3.74	93.3	1017	—	18.1	184	—	—	32.1	326	—	—
b	Wheat	Germinated	3.74	90.6	987	30	14.3	144	40	133	—	—	—	—
c	Wheat	Germinated	3.74	85.6	932	85	6.5	61	123	145	34.2	319	7	8
2a	Wheat	Ungerminated	2.90	93.8	949	—	13.2	125	—	—	32.2	306	—	—
b	Wheat	Germinated	2.90	90.2	913	36	11.5	105	20	56	—	—	—	—
c	Wheat	Germinated	2.90	87.3	883	66	6.6	58	67	102	31.8	281	25	38
3a	Wheat	Ungerminated	2.58	94.1	888	—	13.4	119	—	—	31.6	281	—	—
b	Wheat	Germinated	2.58	88.9	858	30	6.6	57	62	207	32.9	282	−1	−3
4a	Hulled barley	Ungerminated	2.21	95.6	982	—	14.8	145	—	—	—	—	—	—
b	Hulled barley	Germinated	2.21	89.7	921	61	12.6	116	29	48	—	—	—	—
5a	Hulless barley	Ungerminated	2.99	93.3	760	—	15.6	119	—	—	18.5	141	—	—
b	Hulless barley	Germinated	2.99	88.4	720	40	12.6	91	28	70	20.4	147	−6	−15
6a	Rye	Ungerminated	2.83	87.5	456	—	14.4	66	—	—	19.8	90	—	—
b	Rye	Germinated	2.83	81.4	424	32	13.1	55	11	34	21.3	90	0	0
7a	Corn	Ungerminated	1.60	91.0	291	—	12.8	37	—	—	—	—	—	—
b	Corn	Germinated	1.60	89.2	285	6	11.8	33	4	67	—	—	—	—
8a	Peas	Ungerminated	3.33	90.4	458	—	7.2	33	—	—	—	—	—	—
b	Peas	Germinated	3.33	86.7	440	18	7.9	35	−2	−11	—	—	—	—

TABLE II

AMIDE NITROGEN IN NON-PROTEIN NITROGEN OF WHEAT SEEDS AND IN TOTAL NITROGEN OF SEEDLINGS

Sample No.	Condition of seed	Non-protein nitrogen of seeds		Amide N of seed non-protein nitrogen			Total nitrogen of seedlings		Amide N of seedling total nitrogen		Mgm. of amide N produced from protein of seeds in germinating
		% of T.N. of dry seeds	Mgm. per 1000 seeds	% of non-protein N	Mgm. per 1000 seeds	Mgm. increase in germinating	% of T.N. of dry seeds	Mgm. per 1000 seeds	% of T.N. of seedlings	Mgm. per 1000 seeds	
1a	Ungerminated	6.7	73	17.0	12	—	—	—	—	—	—
c	Germinated	14.4	157	13.4	21	9	8.2	90	10.2	9	18
2a	Ungerminated	6.2	63	13.5	9	—	—	—	—	—	—
c	Germinated	12.7	128	12.7	16	7	10.3	104	9.9	10	17

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ALBERTA BITUMEN

I. THE COMPOSITION OF BLOWN ALBERTA BITUMEN[1]

By Morris Katz[2]

Abstract

The relation between the physical consistency and chemical composition of a series of blown asphalts from Alberta bitumen has been studied. The amounts of asphaltous acids and anhydrides decrease on blowing, with rise in fusing point of the bitumen. The amounts of oily constituents and resins also decrease but the asphaltene content increases. The molecular weights of the resins increase from 733 to 1012 during blowing. The asphaltenes also show a pronounced increase in molecular weight from 2219 to 4690. Only traces of oxygen are found in the products blown for 6 to 8.5 hr. at 270° C.; the amount however increases in the later stages of blowing to a maximum of 1.88%. The oily constituents do not contain oxygen, which is distributed mainly in the resin fractions, with smaller amounts occurring in the asphaltene fractions. The sulphur and nitrogen in the bitumen are distributed principally among the resins and asphaltenes. The essential process in blowing bitumen with air is one of condensation by removal of some hydrogen to form water, followed by polymerization, assisted by the sulphur in the bitumen, to products of higher molecular weight. Products similar in composition and physical properties to commercial grades of mineral rubber have been prepared from Alberta bitumen by blowing.

Introduction

The present work was done as part of an investigation of the blowing of bitumen separated from the bituminous sand of Alberta, the immediate objective of the work being to secure from this semi-fluid natural bitumen products suitable for use as "mineral rubber."

It has been known for many years that marked changes may be induced in the physical properties of soft asphalts or residual oils derived from asphaltic, semi-asphaltic or non-asphaltic petroleums, by blowing with air at elevated temperatures. In 1876 W. P. Jenney (6) obtained a patent for treating sludge oil, resulting from petroleum refining, with a current of air at 250° C. Schall (19) proposed the use of oxygen for blowing paraffin, vaseline and heavy mineral oils at 140–150° C., in the presence of insoluble manganese compounds. Salathé (18) patented a process in which heated air was used for blowing asphalts either alone or in the presence of manganese dioxide or litharge. In 1894 Byerley (2) obtained asphalts by drawing air through petroleum residues at temperatures between 400° and 600° F. Soft grades, fusing under 200° F., were prepared by blowing for three days at 400° F., whereas the harder grades were produced by blowing for four to five days at 500° F. This method was used to manufacture blown asphalts on a commercial scale. Similar processes were developed for mixtures of petroleum residues and refined Trinidad asphalt or Gilsonite. Mixtures of air, carbon dioxide and steam were advocated for blowing the asphalt. However, Schreiber (20) showed that treatment with steam or carbon dioxide at

[1] *Manuscript received February 8, 1934.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

[2] *Chemist, National Research Laboratories, Ottawa.*

280° C. left mineral oils practically unchanged, whereas air treatment caused a marked increase in the percentage of material insoluble in benzene. Although numerous patents were taken out involving the use of catalysts in blowing asphalts, Marcusson (14) expressed the opinion that the blowing process is not accelerated by the presence of a catalyst. The various methods in use for the preparation of blown asphalts have been reviewed by Abraham (1).

The chemistry of asphalts is extremely complex and but few data throwing light on their constituents are to be found in the literature. According to Marcusson (12, 13) asphalts may be broadly considered as consisting of a mixture of four groups of compounds; asphaltic acids and anhydrides, asphaltenes, resins and oily constituents. The methods of separating these components from asphalt, as developed by Marcusson, depend upon the fact that the acids and anhydrides are readily removed from a benzene solution of the asphalt by saponification with alcoholic potash solution and washing with water. The asphaltenes may then be precipitated from the benzene layer by adding petrolic ether. The filtrate contains the oils and resins, which are separated by treating the mixture with fuller's earth which adsorbs the resins and thus renders them insoluble in petrolic ether. The resins are then extracted with chloroform or benzene, and the oils with petrolic ether.

Some data are available on the composition of the oil fraction from asphalts. In 1898 Richardson (15) obtained a brown oil by distillation of Trinidad asphalt which on fractionation at a pressure of 60 mm. in an atmosphere of carbon dioxide yielded fractions indicating the presence of members of the $C_n H_{2n}$, $C_n H_{2n-2}$, and $C_n H_{2n-4}$ series. Mabery (9) in 1917 fractionated an oil obtained by the distillation of Gilsonite and Grahamite in an iron pipe at 400° C. and also showed the presence of hydrocarbons of the $C_n H_{2n}$ and $C_n H_{2n-2}$ series. In 1921 Krieble and Seyer (8) reported the results of a careful investigation of the oil fraction from a sample of Alberta tar sand. The sand was extracted with petrolic ether and the mixture of oil and resins thus obtained was treated with fuller's earth. The oil fraction was recovered by distilling off the petrolic ether. About 7.5 litres of this oil was obtained by extraction and subjected to fractional distillation under reduced pressure. A number of hydrocarbons were obtained corresponding to the series $C_n H_{2n}$, $C_n H_{2n-2}$, and $C_n H_{2n-4}$. The compounds were saturated and from a consideration of the molecular refraction it was deduced that they probably possessed ring structures of the mono-, di- and tri-cyclic naphthene type.

The resins and asphaltenes are derived from the oily constituents of the asphalt by condensation and polymerization and also by oxidation. The resins represent the intermediate product in the transformation of the oily constituents to asphaltenes. Their molecular weights are always higher than those of the oils from which they have been derived. Those isolated from blown Alberta bitumen contain oxygen, although the resins from the unblown material are free from oxygen. Marcusson and Picard (14) found that the resin content of a petroleum residue decreased on blowing with air

at 120–130° C. from 14.5% to 3.0%, whereas the asphaltenes increased from 17.5 to 34%. Further blowing at 170° C. increased the asphaltene content to 42.5%, the percentage of resins remaining substantially unchanged at 2.5%. The oily constituents decreased from 68 to 55%.

Sakhanov (Sachenen) (16, 17) investigated the properties of resins and asphaltenes derived from petroleum and its products. The resins contain oxygen and sulphur and are completely soluble in gasoline. Their mean molecular weight and specific gravity (between 1.01 and 1.04) are higher than those of the corresponding oil fractions from which they have been derived. These resins may be liquid, semi-liquid or solid, depending upon the molecular weight. According to Sakhanov, they have a highly developed polycyclic structure of the general formula $C_n H_{2n-m} O_p$ in which n may be 19–55, m 9–33, and p 2–3. They form true solutions, imparting an intense coloration to the solvent. The asphaltenes possess much higher molecular weights than the resins, although structurally these substances appear to be closely related. Sakhanov determined the molecular weight of asphaltenes isolated from various types of petroleum and obtained values of 5000 to 6000 by the cryoscopic method in benzene.

Recently, Mack (11) investigated the viscosity of asphaltenes derived from a blown Mexican asphalt with a softening point of 64° C. Solutions of asphaltenes in petrolenes, *i.e.*, mixtures of oil and resins, yielded values for relative viscosity which increased with falling temperature and also with increasing concentration. By using the relation log. relative viscosity = ckM, where c is the concentration in weight per cent, k, a constant, and M, the molecular weight, Mack obtained an average molecular weight of 1800 for the asphaltenes, from the relative viscosities at 120° C. At 25° C. values of 3480 to 4000 were obtained, and at 0° values as high as 5160 with asphaltenes from the same sample of blown asphalt. Mack ascribes this behavior to the fact that the asphaltenes are in true solution at 120° C. and that association of the molecules, due to their polar nature, takes place at lower temperatures. From the viscosity of sols in benzene, molecular weights of 1794 and 2312 were obtained at 25° and 7° C. respectively.

Chemical Composition of Blown Alberta Bitumen

In the present work, the writer has attempted to show the relation beween the physical consistency of a series of blown asphalts from Alberta bitumen, and their chemical composition. Starting with a product fusing at 34° C. (*R* and *B* method) as the raw material a series of blown products was prepared ranging in softening point from 60 to 169° C. The various bitumen samples were separated into the four main components, namely: (i) the asphaltous acids and anhydrides, (ii) asphaltenes, (iii) resins, and (iv) oily constituents. The amounts of acids and anhydrides decreased with rise in softening point of the bitumen from 1.85 to 0.42%. This is to be expected since at the blowing temperature used, 270–275° C., the saponifiable matter would be converted to unsaponifiable substances resembling asphaltenes. According to Abraham (1) this takes place above 200° C. The asphaltenes

increased from 24.2 to 57.8% in the bitumen of highest fusing point. This change appeared to take place more rapidly in the early stages of blowing, since bitumen fusing at 104° C. was found to contain 47.8% of asphaltenes. The amounts of oily constituents decreased to the same extent, from 55.0% in the raw bitumen to 37.8% in that fusing at 104° C., and finally to 29.1% in the material fusing at 169° C. The amounts of the resins, on the other hand, appeared to remain constant after decreasing from 15% in the raw bitumen to about 9% in the blown product fusing at 104° C. Blown bitumen fusing at 133°, 151° and 169° C. contained 8.4, 7.8 and 8.07% of resins respectively.

Although the hardening which takes place when Alberta bitumen is subjected to heat and air treatment may be initially ascribed to the decrease in the content of oily constituents and their conversion to asphaltenes of higher molecular weight, the later stages of this transformation to a material of high fusing point is no doubt due in large measure to the polymerization of the asphaltenes. Evidence in support of this view was obtained from data on the molecular weight of the oils, resins and asphaltenes extracted from the various bitumen samples. The oily constituents varied in molecular weight from 473 in the raw bitumen to 493–565 in the blown bitumen, the molecular weight decreasing with rise in fusing point. The molecular weight of the resins was 733 in the unblown bitumen, and in the blown samples it varied from 874 to 1012. The molecular weights of the asphaltenes were determined by Rast's method in camphor, since cryoscopic measurements in benzene gave high values similar to those obtained by Sakhanov. Furthermore, there was some separation of the asphaltenes from blown samples into two phases at the freezing point of benzene. For this reason the writer is of the opinion that the molecular weights of asphaltenes in benzene, as determined by the cryoscopic method, are probably much higher than the true values. The asphaltenes in the unblown bitumen had a mean molecular weight of 2219. This value was increased slightly to 2560 in the asphaltenes from bitumen blown to a fusing point of 104° C. Thereafter the molecular weight of the asphaltenes rose rapidly with increase in softening point of the bitumen, a mean value of 4690 being obtained in the fraction isolated from bitumen fusing at 169° C. The data in Table I indicate the extent of poly-

TABLE I

ASPHALTENES IN ALBERTA BITUMENS OF VARIOUS CONSISTENCIES

Description of bitumen	Fusing point (*R* and *B* method), ° C.	Asphaltenes, %	Molecular weight of asphaltene fraction	Softening points or sintering points of asphaltenes, ° C.
Original bitumen	34	24.2	2219	156–162
Blown bitumen	104	47.8	2560	255–263
Blown bitumen	133	48.4	3130	304–308
Blown bitumen	151	53.4	4225	355–340
Blown bitumen	169	57.8	4690	360–368

merization of the asphaltenes with rise in fusing point of the bitumen, as well as the relation between the softening or sintering points of the asphaltenes and their molecular weight. It is evident that the molecular size and percentage of asphaltenes present in blown asphalts exert a marked influence on their physical consistency.

Ultimate Composition of Blown Alberta Bitumen

The behavior of asphalts and asphalt base oils on oxidation is in marked contrast to that of paraffins. Whereas the latter are readily converted by oxidation into mixtures of fatty acids of about one-half the average number of carbon atoms in the molecule, involving the combination of considerable amounts of oxygen (7), this is not the case with asphalts and asphalt base oils which contain only small amounts of oxygen even after blowing for several days at temperatures of 200 to 275° C. The effect on Alberta bitumen of oxidation with air at 270 to 275° C. was similar to that on asphalts. As shown

TABLE II

OXYGEN CONTENT OF BLOWN ALBERTA BITUMEN

Period of blowing (hr.)	0	4	6	8.5	14	—
Fusing point, ° C.	34	79	104	133	151	169
Oxygen in product, %	Nil	Nil	Trace	Trace	0.60	1.88

in Table II, only 0.6 % of oxygen was found in bitumen after blowing for 14 hr. at 270° C. at the rate of 6 cc. of air per minute per gram of material. After raising the softening point of the bitumen to 169° C., 1.88% of oxygen was found to have combined with the material.

The oxygen was distributed mainly in the resin fraction of the bitumen, and to a lesser extent in the asphaltenes. The oily constituents were free from oxygen.

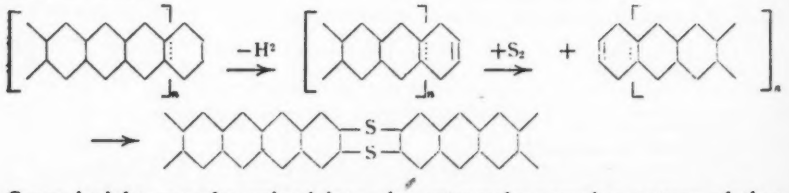
TABLE III

ULTIMATE COMPOSITION OF ALBERTA AND COMMERCIAL BITUMENS

Description of bitumen	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen, %
Alberta bitumen, f.p. 34° C.	85.43	9.52	4.52	0.57	Nil
Blown Alberta bitumen, f.p. 151° C.	84.4	9.17	5.23	0.60	0.6
Blown Alberta bitumen, f.p. 169° C.	83.32	8.99	5.25	0.56	1.88
Trinidad asphalt (15, p. 29)	82.33	10.69	6.16	0.81	—
Bermudez (15, p. 29)	82.88	10.79	5.87	0.75	—
Commercial grade blown asphalt, f.p. 149° C.	84.83	9.06	4.93	0.63	0.55
Commercial grade blown asphalt, f.p. 168° C.	85.16	8.98	3.51	1.07	1.28

It is of interest to compare the ultimate composition on an ash-free basis of the raw bitumen with that of the highly blown product and other natural and blown asphalts. The blown Alberta bitumen is quite similar in composition to the corresponding commercial grades of blown asphalt used as mineral rubbers. The main difference between the bitumen fusing at 169° C. and the commercial product fusing at 168° C. lies in the somewhat lower sulphur content of the latter. Throughout this study corresponding determinations of chemical composition and physical properties were made, for purposes of comparison on two well known commercial grades of bitumen used in the rubber industry (*c.f.* Table III).

The results obtained from the ultimate analysis of a series of blown bitumen products and their corresponding chemical components, *i.e.*, the oily constituents, resins and alphaltenes, indicate that condensation and polymerization are the essential transformations which take place during blowing or oxidation of asphalts at elevated temperatures. The oxygen of the air probably acts as a catalyst. A small amount of hydrogen (less than 0.5%) is liberated during the condensation of the oily constituents to asphaltenes, through the intermediate resin stage, and it combines with the oxygen to form water. Unsaturated compounds formed at this stage are rapidly polymerized to products of higher molecular weight. The substances formed are saturated and cyclic in nature, since unsaturation determinations with Waller's solution (21) on the oils, resins and asphaltenes yielded zero values in nearly every case. It appears therefore that polymerization takes place through the scission of the ring compounds in such a manner that a small fragment of the molecule, or two atoms of hydrogen, are split off and the remainder unites with a similar molecule. This may take place through the agency of sulphur and, to a lesser extent, oxygen. Sulphur evidently plays an essential part in the polymerization, since the resins contain about 6% and the asphaltenes 7% of sulphur. Even higher amounts of sulphur have been found in the asphaltenes from Trinidad asphalt (13). The greater part of the sulphur present in the original bitumen is held in combination in the asphaltene fraction. The mechanism of the polymerization may be represented as follows.



Some insight may be gained into the extremely complex nature of these substances by calculating the number of carbon atoms present in the molecule in the asphaltene fraction of Alberta bitumen. An average molecule in the asphaltenes from raw bitumen consists of approximately 140 carbon atoms, 168 hydrogen atoms and 4 sulphur atoms. In blown bitumen, the asphaltene molecule may consist of double this number of atoms.

Experimental

The Sample

The Alberta bitumen used in this work was part of a shipment made to the National Research Laboratories by Dr. K. A. Clark of the Research Council of Alberta. The material had been extracted from tar sand by Dr. Clark, according to methods developed by him (4). Before use, the bitumen was carefully dehydrated and found to contain only about 0.5% of water. The ash content of various samples was from 4.3 to 4.6%. Analysis of this ash indicated that it was a finely divided clay of the composition shown in Table IV.

TABLE IV

COMPOSITION OF ASH OF ALBERTA BITUMEN

SiO_2, %	Al_2O_3, %	Fe_2O_3, %	CaO, %	MgO, %	Total, %
56.1	25.0	16.1	1.6	0.9	99.7

The bitumen, after dehydration, possessed a fusing point of 34° C.

Apparatus

The apparatus used for blowing the bitumen with air is shown in Fig. 1. The sample was placed in an iron still-pot, *A*, equipped with a tightly fitting cover plate, a blow coil, *F*, a stirrer, and a thermometer well, *D*. A reflux condenser, *E*, fitted into the cover plate, the upper portion of the condenser being filled with glass beads. The still-pot was supported by a small tripod in an oil bath, *B*, containing an oil, of high flash point, known as Superheat valve oil. The bath was heated by gas burners and the temperature was controlled by means of the mercury regulator, *C*, operating on the gas feed line. The regulation of temperature was effected by the expansion and contraction of the air entrapped above the mercury column in the inverted bulb. Air was sent into the surge tank, *K*, and was measured with the wet-test meter, *H*, and the calibrated capillary flow meter, *G*, before passing through the bitumen.

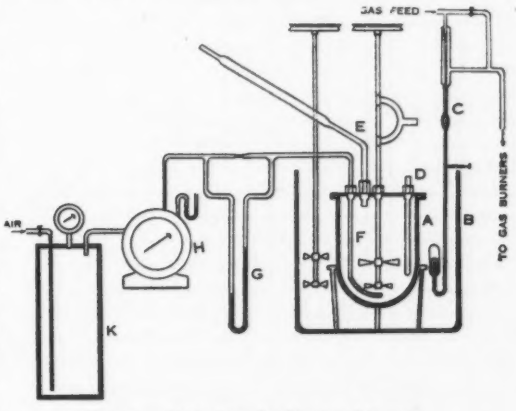


FIG. 1. *Apparatus for blowing bitumen.*

Effect of Air at 270 to 275° C.

Preliminary experiments without a reflux condenser indicated that from 25 to 45% of a yellow to reddish brown oil may be removed from the bitumen

by blowing with air at elevated temperatures. However, the residue was found to be very brittle, insoluble in rubber and lacking in ductility. On the other hand, products possessing a high dispersive power in rubber and readily soluble in solvents were prepared by condensing the greater part of the volatile oil formed during blowing and returning it as reflux to the contents of the still. Some of the results have been presented in Table V. The rate of blowing has been calculated on the basis of the number of cubic centimetres passed through per minute per gram of sample. The fusing point of the blown bitumen was determined by the ring and ball method according to A.S.T.M. specifications. At the temperature and rate of blowing employed, the fusing point of Alberta bitumen rises with increase in the time of blowing according to a linear relation, until a fusing point of 133° C. is reached. Beyond this stage the fusing point increases less rapidly with the time of blowing (Fig. 2). The fusing point may also be increased by either increasing the rate of blowing or raising the temperature. However it is impracticable to blow Alberta bitumen at temperatures above 285° C., since on several occasions when this was attempted a rapid evolution of gas took place with a sudden rise in the temperature of the bitumen. This reaction must be due to oxidation since it ceased when the air stream was turned off.

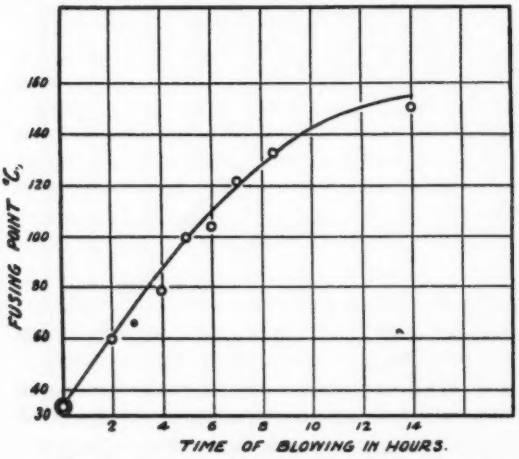


FIG. 2. *Increase of fusing point with time of blowing Alberta bitumen.*

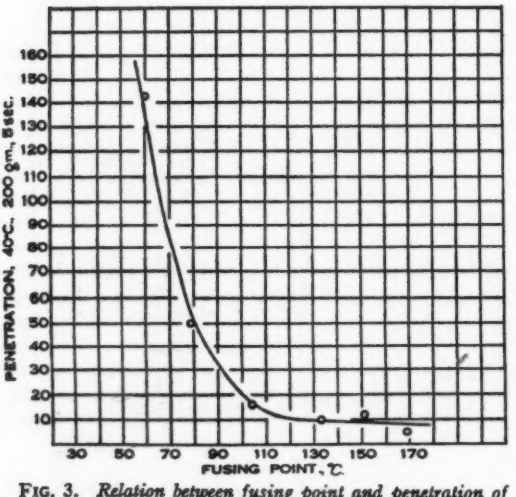


FIG. 3. *Relation between fusing point and penetration of blown Alberta bitumens.*

The effect of air blowing on the penetration of Alberta bitumen is shown in Table VI. The unblown material is highly susceptible to change in temperature. Air blowing reduces this susceptibility. The penetration at a given temperature

decreases very rapidly with rise in fusing point in the early stages of blowing. Beyond a fusing point of 104° C. the effect on penetration is quite small (Fig. 3).

TABLE V

EFFECT OF BLOWING WITH AIR ON FUSING POINT OF ALBERTA BITUMEN

Sample no.	Weight of sample, gm.	Temperature of oil bath, ° C.	Rate of blowing, cc. of air per min. per gm. of sample	Period of blowing, hr.	Volatile oil distilled out, %	Fusing point of product, °C.
36	1500	270	6.0	2.0	3.0	60
35	1500	270	6.0	4.0	4.0	79
37	1500	270	6.0	5.0	5.7	100
39	1500	270	6.0	6.0	8.7	104
38	1500	270	6.0	7.0	11.2	122
40	1500	270	6.0	8.5	11.5	133
31	4670	270	6.0	14.0	6.5	151
33	4619	275	8.0	13.5	11.1	169
22	400	275	2.0	21.0	11.0	168
25	400	275	6.0	8.0	10.0	156
*26	400	275	6.0	9.0	9.5	150
†27	400	275	6.0	6.5	3.5	157
‡24	400	275	4.0	6.0	6.3	156

**This sample was blown in two stages of 5½ and 3½ hr., necessitating re-heating of the bitumen after the first stage to 275° C.*

†Sample contains 15% Gilsonite.

‡Sample contains 1% anhydrous stannic chloride.

TABLE VI

PENETRATION OF ALBERTA BITUMEN

Sample no.	Fusing point, ° C.	Penetration					
		40° C. 100 gm., 5 sec.	40° C. 200 gm., 5 sec.	25° C. 100 gm., 5 sec.	25° C. 200 gm., 5 sec.	5° C. 100 gm., 5 sec.	5° C. 200 gm., 5 sec.
Unblown	34	Too fluid	Too fluid	184.5	250.5	23.5	47.0
36	60	95.7	143.5	15.5	27.3		
35	79	21.3	49.7	6	10.2		
39	104	6.8	15.7	3	5.4		
40	133	2.2	9.7	Too viscous	0.0		
31	151	0.0	11.8	Too hard			
33	169	0.0	5.0	Too hard			
Hard Hydrocarbon	149	3.7	7.0	1.0	4.5		
Genasco	168	2.0	3.8	0.0	2.8		

Method of Separating the Components of Bitumen

The following procedure was adopted for the determination of asphaltous acids and anhydrides, since the method of Marcusson was found to lead to the formation of troublesome emulsions.

1. *Saponifiable matter.* About 5.00 gm. of substance was dissolved in 50 cc. of benzene by boiling under a reflux condenser. The solution was filtered and 25 cc. of normal alcoholic potassium hydroxide added and the mixture refluxed for about 1½ hr. The mixture while still warm was transferred to a separatory funnel containing 150 to 200 cc. of 40% ethyl alcohol in hot water; 25 cc. of a 5% solution of potassium chloride was added, and finally 100 cc. of benzene. The contents of the separatory funnel was agitated vigorously for several minutes and allowed to stand until separation took place. The benzene layer was removed and worked up as under Section 2. The alcoholic soap solution was drawn off and exhausted with small portions of benzene to remove unsaponifiable matter. The benzene layer was similarly exhausted with 40% ethyl alcohol to remove saponifiable matter. The alcoholic extracts were combined and the soap solution evaporated to a volume of about 100 cc. The asphaltous acids and anhydrides were liberated by the addition of dilute hydrochloric acid and then exhausted with benzene. The benzene extract was evaporated to a small bulk and the evaporation of the solvent completed at 100° C. The asphaltous acids and anhydrides were dried to constant weight. In appearance they were brown masses, the material from the raw bitumen and sample No. 39 being somewhat resinous and tacky, while that from the remaining samples was solid.

In several instances during the above determinations of saponifiable matter, an intermediate layer was formed in the separatory funnel, between the benzene and alcohol-water layers. This consisted of emulsified material which was rather difficult to break up by the further addition of either water or benzene. It was therefore evaporated to about 25 cc. and treated with 75–100 cc. of 40% aqueous alcohol, thus breaking the emulsion. The soap solution was exhausted with small amounts of benzene and then combined with the main alcoholic extract.

The asphaltenes, resins, and oily constituents were determined according to the methods of Marcusson (12).

2. *Asphaltenes.* After removing the saponified material the original benzene layer was evaporated to a volume of 25 cc. and the solution poured into 300 cc. of petrolic ether, b.p. 35°–60° C. The mixture was allowed to stand for several hours and the precipitated asphaltenes filtered. The precipitate was carefully washed with petrolic ether, dried at 100° C. and weighed. The filtrate and washings were combined and examined further.

Resins. The combined petrolic ether filtrates were evaporated to a volume of 25 to 30 cc. and then distributed over 50 gm. of fuller's earth or 100 gm. of silica gel. The oily constituents were removed by extraction of the material with petrolic ether, b.p. 35–60° C. The adsorbed resins were then removed by extraction with chloroform. The chloroform was distilled off and the resins dried at 100° C. and weighed.

Oily Constituents. The petrolic ether extract obtained as above was evaporated on the water bath and the solvent removed. The oil was finally kept at

a temperature of 100° C. for several hours to remove traces of solvent and then weighed. In cases where the first extract was dark in color, it was concentrated to 25 cc. and again treated with fuller's earth.

The components of natural and blown Alberta bitumen were separated according to this procedure and compared with two blown asphalts which are widely used in the rubber industry as mineral rubbers. The data are presented in Table VII.

TABLE VII

COMPONENTS OF NATURAL AND BLOWN ALBERTA BITUMEN

Bitumen	Asphaltous acids and anhydrides, %	Asphaltenes, %	Resins, %	Oily constituents, %	Ash, %
Raw bitumen	1.85	24.2	15.0	55.0	4.3
Bitumen, f.p. 104° C.	0.87	47.8	9.3	37.8	4.5
Bitumen, f.p. 133° C.	0.64	48.4	8.4	39.2	4.2
Bitumen, f.p. 151° C.	0.46	53.4	7.8	34.9	4.62
Bitumen, f.p. 169° C.	0.42	57.8	8.07	29.1	4.38
Hard Hydrocarbon, 149° C.	0.25	45.9	13.57	40.2	0.21
Genasco, f.p. 167° C.	0.45	47.4	12.2	39.8	0.51

The amounts of asphaltous acids and anhydrides decrease with rise in fusing point of the bitumen from an original value of 1.85 to 0.42% in the material of highest fusing point. The asphaltenes show a progressive increase in amount with rise in fusing point of the bitumen. The amount of resins, on the other hand, decreases from an initial value of 15.0% to about 8% in the material fusing in the temperature range 133°–169° C. The percentage of oily constituents decreases from 55.0 in the unblown bitumen to 29.1 in that with a softening point of 169° C. Both the Hard Hydrocarbon and Genasco mineral rubbers contain a higher amount of oily constituents and a lower amount of asphaltenes than comparable samples from blown Alberta bitumen. The asphaltenes from the two commercial grades of mineral rubber, on the other hand, soften at much higher temperatures and possess higher molecular weights than those extracted from corresponding grades of blown bitumen.

The asphaltenes from the different Alberta bitumen samples form a well defined series, the lowest number of which softens over the temperature range 156–162° C. and the highest at 360–368° C. as shown in Table VIII. The sintering points were obtained by means of an electrical melting point apparatus, at least three determinations being carried out on each sample.

TABLE VIII

RANGE OF SINTERING POINTS OF ASPHALTENES

Asphaltenes precipitated from:—	Softening or sintering point range, ° C.	Remarks
Raw bitumen, f.p. 34° C.	156–162	
Bitumen, f.p. 104° C.	255–263	Cracking began at 276–288° C. Brown oil distilled off.
Bitumen, f.p. 133° C.	304–308	Cracking set in at 312° C. Brownish oil distilled off.
Bitumen, f.p. 151° C.	335–340	Brownish oil began to distil at 340–345° C.
Bitumen, f.p. 169° C.	360–368	Reddish brown oil given off at this temperature.
Hard Hydrocarbon, f.p. 149° C.	387–395	Cracking set in at about 395–405° C. with evolution of reddish brown oil.
Genasco, f.p. 167° C.	385–395° C. Melts rapidly at 395° C.	Cracking set in at 395–405° C. with evolution of reddish brown oil.

TABLE IX

IODINE NUMBER OF BITUMEN COMPONENTS

Sample	Iodine no.
Resin from natural Alberta bitumen	6.2
Asphaltenes from Hard Hydrocarbon	1.1
Asphaltenes from Genasco	19.5

Iodine Number

Unsaturation determinations were carried out on the oil, resin and asphaltene fractions listed in Table VII, using Waller's solution. Zero values were obtained for the iodine number in all cases except those shown in Table IX.

Properties of Oils, Resins and Asphaltenes

The oils from the natural and blown Alberta bitumen were yellow to light red, while those from Hard Hydrocarbon and Genasco were darker. The refractive indices of the oils are shown in Table X. The refractive index of the oils from the Hard Hydrocarbon and Genasco could not be measured accurately because of their color.

TABLE X

REFRACTIVE INDICES OF OILS FROM NATURAL AND BLOWN ALBERTA BITUMEN

Oil from:	n^{25} ° C.
Raw bitumen	1.4999 ($d^{25}=0.940$)
Bitumen No. 39	1.4986
Bitumen No. 40	1.4982
Bitumen No. 31	1.4975
Bitumen No. 33	1.4964

The resins from raw Alberta bitumen are soft and tacky and reddish brown. Those from blown bitumen are solid, melt at about 80° to 90° C. and are also red-brown. They are readily soluble in benzene, chloroform, carbon tetrachloride and petrolic ether, sparingly soluble in hot acetone and insoluble in alcohol. The resins form true solutions and impart an intense coloration, from yellow to red, to these solutions, depending on the concentration. This

color is more intense in the resins of higher molecular weight. The specific gravity of the resins from raw Alberta bitumen is 1.002 at 27° C.

The asphaltenes partake of the nature of hemicolloids. They are readily soluble in benzene, carbon disulphide, chloroform, carbon tetrachloride, but insoluble in petrolic ether, acetone or alcohol. The solutions when dilute, are deep red and in more concentrated solutions, reddish-brown. The coloring power of the asphaltenes is much greater than that of the resins. The solubility of the asphaltenes in benzene decreases with rise in molecular weight. They are apparently insoluble in the rubber hydrocarbon, since solutions of rubber and asphaltenes in benzene when mixed show a separation of the asphaltenes.

This behavior indicates that these two polymeric materials differ basically in structure, the rubber hydrocarbon being essentially built up of long chains, aliphatic in nature, whereas the asphaltenes probably possess a highly developed polycyclic structure.

The density of asphaltenes from raw bitumen at 27° C. is 1.126.

The writer has separated the oily constituents, resins, and asphaltenes from two kilograms of Alberta bitumen using a large copper Soxhlet extractor. A further examination of the properties of these materials will form the subject of a separate paper.

Molecular Weights of the Oils, Resins and Asphaltenes

The molecular weights of the oils and resins were determined quite readily by the cryoscopic method in benzene. Before determining the molecular weight of the asphaltenes, they were reprecipitated from benzene solution with petrolic ether. Cryoscopic measurements on solutions of asphaltenes in benzene yielded values between 4300 and 5600. The results were not consistent however and on closer examination it was found that a slight separation into two phases took place at the freezing point of the solution.

The asphaltenes were found to yield homogeneous solutions in camphor and the molecular weight of these fractions were therefore carried out according to the method of Rast. The results are presented in Table XI.

TABLE XI

MOLECULAR WEIGHTS OF OILS, RESINS AND ASPHALTENES (DERIVED BY EXTRACTION FROM BLOWN BITUMEN)

Origin of fraction	Mol. wt. of oily constituents (Cryoscopically in benzene)	Mol. wt. of resins (Cryoscopically in benzene)	Mol. wt. of asphaltenes (Rast)	Origin of fraction	Mol. wt. of oily constituents (Cryoscopically in benzene)	Mol. wt. of resins (Cryoscopically in benzene)	Mol. wt. of asphaltenes (Rast)
Raw bitumen, f.p. 34° C.	473	733	2219	Bitumen, f.p. 169° C.	493	930	4690
Bitumen, f.p. 104° C.	560	874	2560	Hard Hydrocarbon,			
Bitumen, f.p. 133° C.	565	956	3130	f.p. 149° C.	700	1497	4870
Bitumen, f.p. 151° C.	497	1012	4225	Genasco, f.p. 168° C.	684	1410	5160

The molecular weights of the oils in bitumen increase from 473 in the raw bitumen to 565 in bitumen fusing at 133° C., followed by a drop to 493 in bitumen fusing at 169° C. The oils from Hard Hydrocarbon and Genasco are of considerably higher molecular weight than those taken from analogous Alberta bitumen samples. The average molecular weights of the resins increase from 733 to 1012 owing to polymerization. The resins from the two commercial samples are of considerably higher molecular weight as in the case of the oils.

The asphaltenes are gradually polymerized by the air blowing treatment accorded the bitumen at 270 to 275° C. The average molecular weight of the lowest fraction is 2219 and this value increases with rise in fusing point of the blown bitumen to an average value of 4690 in the asphaltene fraction from the sample fusing at 169° C. The sintering points of the asphaltenes increase with rise in molecular weight in a manner which suggests that these substances form a series of complex polymers (Table VIII).

Ultimate Analysis of Blown Bitumen, Oily Constituents, Resins and Asphaltenes

A series of samples of blown bitumen, and the oily constituents, resins and asphaltenes, were subjected to full elementary analysis in order to determine the effect of the air and heat treatment. Determinations were made of carbon, hydrogen, sulphur and nitrogen. Carbon and hydrogen were determined in an electrical combustion apparatus. The sulphur was first determined by fusion with sodium peroxide in nickel crucibles according to the A.O.A.C. official method. This was found to check with the Parr bomb method in the case of oils and resins. The blown bitumen samples and the asphaltene fractions, on the other hand, yielded better results when the Parr bomb was used for carrying out the fusions than when the A.O.A.C. open crucible method was used. In the latter case the results were too low owing to difficulty in carrying out a complete fusion. All the solid samples were finely ground in a mortar, prior to analysis, but in those cases where the material was too plastic to be ground, it was found expedient to dissolve the sample in benzene free from sulphur, mix with a little sodium carbonate, dry at 90° C. and then grind and mix with the proper amount of fine sodium peroxide in the Parr bomb.

The nitrogen was determined by the Kjeldahl method.

In comparing the data presented in Table XII, it must be borne in mind that raw Alberta bitumen is not a material of fixed composition; considerable variations may occur in samples extracted from Alberta sands from different localities. What is probably an extreme case of variation in composition may be shown by comparing the data in the table on raw bitumen with the values obtained by Krieble and Seyer (8) on their sample extracted from sand taken from an exposure on the Hangingstone river, three miles from Fort McMurray. Their values for the raw bitumen are:—C, 84.49; H, 11.23; S, 2.73; and N, 0.04%. Nearly all the samples analyzed by Dr. Clark of the Research Council of Alberta contain between 4 and 5% of sulphur (3).

TABLE XII

ELEMENTARY COMPOSITION OF BLOWN BITUMEN (ASH-FREE BASIS)

Description of sample	Fusing point, ° C.	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen by difference	Total
Raw bitumen	34	85.43	9.52	4.52	0.57	Nil	100.04
Bitumen No. 36	60	84.71	9.71	5.30	0.56	Nil	100.28
Bitumen No. 35	79	84.65	9.54	5.34	0.56	Nil	100.09
Bitumen No. 37	100	84.90	9.13	5.34	0.62	Nil	99.99
Bitumen No. 39	104	84.50	8.88	5.42	0.73	Trace	99.53
Bitumen No. 38	122	85.27	8.87	5.37	0.65	Trace	100.16
Bitumen No. 40	133	84.60	9.05	5.25	0.65	Trace	99.55
Bitumen No. 31	151	84.4	9.17	5.23	0.60	0.6	100.0
Bitumen No. 33	169	83.32	8.99	5.25	0.56	1.88	100.0
Hard Hydrocarbon	149	84.83	9.06	4.93	0.63	0.55	100.0
Genasco	168	85.16	8.98	3.51	1.07	1.28	100.0

The samples in Table XII were prepared from the same batch of raw material contained in a shipment made by Dr. Clark, the variation in elementary composition of which may be assumed to be quite small. As the melting point of the bitumen rises with continued blowing, there occurs a drop in the carbon and hydrogen content. The sulphur and nitrogen content appear to be unaffected by this treatment. The net result is a gradual combination with oxygen. This oxidation of the asphalt is appreciable when the melting point is about 150° C. or higher. The over-all experimental error involved in the analyses is about $\pm 0.3\%$ and it is therefore difficult to detect the presence of oxygen in amounts below 0.5%. However it would appear from the analyses of the resins (Table XIV) that the bitumen melting at 104° C. contains only a small amount of oxygen. The resins from this sample contain 1.4% of oxygen. No oxygen appeared to be present in the oil and asphaltene fractions. Since the resin fraction was 9.3% of the bitumen, the total oxygen in this sample is about 0.13%. The hydrogen removed from the bitumen during blowing is converted in part into water by combination with oxygen from the air. Some of the hydrogen however is used up in enriching the oil formed during prolonged blowing when the melting point of the bitumen

TABLE XIII

ELEMENTARY COMPOSITION OF OILY CONSTITUENTS

Description of sample	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %
Oily constituents from raw bitumen	86.99	11.02	2.08	0.28
Oily constituents from bitumen No. 39	86.46	10.49	3.60	0.18
Oily constituents from bitumen No. 40	85.75	10.63	3.76	0.12
Oily constituents from bitumen No. 31	86.46	11.10	2.74	0.10
Oily constituents from bitumen No. 33	86.36	11.02	2.69	0.06
Oily constituents from Genasco	86.76	10.70	2.46	0.15
Oily constituents from Hard Hydrocarbon	85.30	10.60	4.30	0.11

rises above 133° C., since the hydrogen content of the oily constituents shows an increase in samples 31 and 33 (Table XIII).

It is of interest to observe that the bitumen with a fusing point of 151° C. agrees very closely in elementary composition with Hard Hydrocarbon, a popular grade of mineral rubber having a similar fusing point. The elementary composition of Genasco, another commercial grade of mineral rubber, differs from its analogue, sample No. 33, in having a lower sulphur content but a higher nitrogen content (Table XII).

A comparison of the data in Tables XIII, XIV and XV, shows the distribution of sulphur, nitrogen, and oxygen in the various components which make up the complex material known as bitumen, *i.e.*, the oils, resins and asphaltenes. The largest portions of the sulphur and nitrogen are present in the asphaltene fractions, with correspondingly smaller amounts in the resins and oils. The oils are free from oxygen, whereas the resins of the blown samples contain above 1% of oxygen, increasing to 2.65% in the most highly blown material. In the case of the asphaltenes, only those fractions extracted from bitumen fusing above 133° C. contain oxygen. The oxygen content then increases with rise in melting point.

TABLE XIV

ELEMENTARY COMPOSITION OF RESINS

Description of sample	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen by difference
Resin from raw bitumen	84.28	9.39	6.02	0.40	Nil
Resin from bitumen No. 39	82.62	9.66	5.62	0.64	1.4
Resin from bitumen No. 40	83.14	9.24	6.01	0.56	1.05
Resin from bitumen No. 31	82.81	9.11	5.67	0.58	1.83
Resin from bitumen No. 33	81.35	9.63	5.87	0.50	2.65
Resin from Hard Hydrocarbon	82.68	8.88	6.70	0.65	1.09
Resin from Genasco	83.12	9.75	4.72	0.89	1.52

TABLE XV

ELEMENTARY COMPOSITION OF ASPHALTENES

Description of sample asphaltenes from:—	Carbon, %	Hydrogen, %	Sulphur, %	Nitrogen, %	Oxygen by difference	Total
Raw bitumen	84.0	8.42	6.58	0.99	Nil	99.99
Bitumen No. 39	83.93	7.93	7.08	1.26	Nil	100.20
Bitumen No. 40	84.11	7.85	6.98	0.96	Nil	99.90
Bitumen No. 31	83.81	7.84	6.94	0.98	0.43	100.0
Bitumen No. 33	82.34	7.76	6.72	1.02	2.16	100.0
Hard Hydrocarbon	84.56	7.81	5.07	0.99	1.57	100.0
Genasco	86.31	7.61	3.24	1.41	1.43	100.0

Both the carbon and hydrogen content of the oils (Table XIII) show a decrease in the early stages of blowing, *i.e.*, polymerization of the oil occurs due to condensation reactions involving the removal of hydrogen, followed

by an increase in the later stages due probably to "cracking" reactions and the formation of products of lower molecular weight. The molecular weights of the oils, as already pointed out, increase from 473 in the raw bitumen to 565 in bitumen fusing at 133° C. followed by a drop to 493 in the sample of highest fusing point.

The essential differences in the composition of the resins compared with the oils are the marked increase in sulphur content and the increasing amount of oxygen present. The resins appear to be quite susceptible to oxidation. Their high sulphur content is no doubt due to the fact that polymerization of the oils to the resin stage has been accomplished in part through the agency of sulphur. The nitrogen content of the resins is appreciably higher thar that of the oils.

The asphaltenes do not appear to be as susceptible to oxidation by blowing with air as the resins. They contain higher amounts of sulphur and nitrogen than the corresponding resin fractions from which they appear to be derived. These elements seem to be in a very stable form of combination since treatment with air for periods up to 14 hr. at 275° C. does not alter to any appreciable extent the proportions present in the asphaltenes. The net result of such treatment is a gradual polymerization of the asphaltenes from a molecular weight of 2219 in the raw bitumen fraction to 4690 in that taken from blown bitumen fusing at 169 ° C.

Acknowledgments

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HEAT CAPACITY MEASUREMENTS ON GELATIN GELS[1]. II.

By W. F. Hampton[2] and J. H. Mennie[3]

Abstract

Measurements of the heat capacity of gelatin gels, prepared from ash-free, isoelectric gelatin, have been extended to cover the temperature range between −180° and 25° C. and the concentration range from 9 to 100% gelatin. Change in the temperature of the gel above the freezing point is found to be accompanied by a heat effect which has not previously been taken into account in calorimetric measurements on gels and which renders the interpretation of the experimental data uncertain.

Introduction

In a previous paper (3) a method for determining the heat capacity of gelatin gels was described and results reported for a 24% gel, dry gelatin and a gel containing 12.5% water. The calculation of unfrozen or "bound" water from such measurements was discussed and a new equation was derived. In the present paper further investigations are reported covering the range of concentration of gelatin gels from 9 to 100% gelatin, over the temperature range from −180° to 25° C.

Experimental

Apparatus

The apparatus and procedure have already been described (3, 4). Only slight modifications were necessary to make the method applicable over the wider range of concentration and of temperature.

The gelatin used was the same "ash-free", isoelectric gelatin supplied by the Eastman Kodak Co. (Lot No. 48). The ash content was 0.04% and the pH was given as 4.85.

The gels were prepared as before by soaking the sheets of gelatin in distilled water, which had been boiled and allowed to cool to room temperature, long enough for the gelatin to take up approximately the desired amount of water. In the case of the more dilute gels the water-soaked material was heated at 50° C. for about 30 min. and finally poured into the container. The container was then rotated in a horizontal position at about 300 r.p.m. until the gel had set. This caused the gel to set around the sides of the container, giving improved heat transfer and also leaving a cylindrical space in the centre of the gel which permitted expansion on freezing.

This method of filling the container was found to be very satisfactory for gels up to about 45% gelatin. The very high viscosity in the sol form of more concentrated gels prevents pouring them readily. Hence, in the case of gels containing more than 45% gelatin, the container was filled by pressing the water-soaked material into it, and the heating at 50° C. was carried out with the sample enclosed in the container. Since in gels of these higher con-

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centrations there is very little expansion during freezing, the procedure of rotating the container as the gel cooled was omitted. The percentage of dry gelatin in the concentrated gels was obtained by cutting portions from various parts of the water-soaked sheets of gelatin immediately they were removed from the water. These were analyzed by heating to constant weight in an oven at 105° C. In all cases duplicate determinations were made and the average taken as the concentration of the gel.

As before, an ice-water mixture was used as the constant temperature bath at 0° C. and a solid carbon dioxide—ether mixture for −78.5° C. Temperatures between these limits were obtained by cooling ether or acetone to the desired temperature and maintaining it at a constant value by dropping in small lumps of solid carbon dioxide at regular intervals. Liquid air was used to give the lowest temperatures, near −183° C. The temperature was always carefully checked by means of the platinum thermometer, since the boiling point of liquid air varies with the nitrogen content.

The arrangement of the bath for temperatures near −130° C. is illustrated in Fig. 1. The bath liquid was obtained by distilling gas machine naphtha and collecting the fraction boiling below 30° C. This was brought to the desired temperature and maintained at this point by blowing liquid air at regular intervals into a very thin copper tube, *P*, suspended in the bath liquid. To make the cooling as efficient and rapid as possible, horizontal disks of copper were soldered to the copper tube, giving a set of vanes projecting into the bath liquid. Conduction of heat into the bath through the copper tube was minimized by fixing it to a short length of Pyrex tubing. This was done by painting the surface of the glass with gold paint, heating to attach the gold firmly to the glass, and depositing electrolytically a layer of copper on the gold. The copper tube was then soldered to the layer of copper on the glass. This arrangement was suspended in the bath liquid so that only the glass tubing projected above the surface. Stirring was effected by means of dry air and the temperature was measured by the calibrated platinum thermometer, *T*.

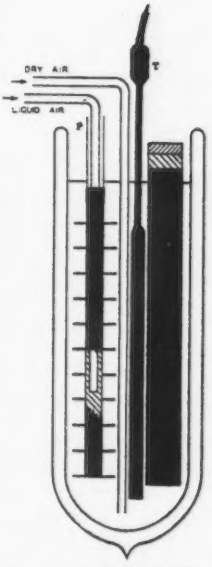

FIG. 1. *Bath for temperatures between −78.5° C. and −180° C.*

It is estimated that with careful manipulation the temperature of the thermostat could be controlled to about 0.2° at − 130° C. and easily to 0.1° at temperatures above −78.5° C.

Results

All heat capacity calculations were made to 25° C. The experimental values for the total heat capacity of container plus gel are given in Table I. The values for the 24% gel and those for the 87.5% gel and dry gelatin down to−78.5° C., which were reported in the previous paper (3), are here repeated

TABLE I

TOTAL HEAT CAPACITY OF CONTAINER PLUS GEL, IN CALORIES

Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity	Initial temp., °C.	Heat capacity
Container alone		Dry gelatin, 7.6590 gm.		87.5% Gel, 9.7415 gm.		67.7% Gel, 10.0525 gm.		61.4% Gel, 8.4173 gm.	
25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0
0.0	−335.6	0.0	−389.5	0.0	−414.4	0.0	−501.4	0.0	−459.3
−26.7	−686.4	−20.2	−687.0	−29.2	−900.1	−4.9	−587.5	−2.1	−493.6
−57.8	−1077.9	−34.9	−909.4	−30.5	−905.8	−5.2	−599.5	−4.0	−533.6
−78.5	−1339.5	−56.2	−1207.2	−52.4	−1238.6	−9.2	−671.0	−5.0	−571.6
−132.6	−1925.5	−78.5	−1523.8	−78.5	−1613.6	−9.4	−672.0	−9.4	−672.3
−184.2	−2371.7	−131.6	−2166.6	−129.6	−2263.2	−23.3	−928.5	−15.0	−780.8
		−184.5	−2678.0	−183.5	−2781.6	−39.5	−1221.5	−32.8	−1075.1
						−78.5	−1849.9	−55.4	−1437.8
						−123.6	−2467.4	−78.5	−1798.7
						−184.1	−3113.0	−130.4	−2494.9
								−183.9	−3041.1
58.1% Gel, 9.1576 gm.		45.3% Gel, 15.1890 gm.		24.0% Gel, 12.2845 gm.		20.9% Gel, 11.9608 gm.		9% Gel, 12.9730 gm.	
25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0	25.0	0.0
0.0	−482.3	0.0	−636.9	0.0	−607.0	0.0	−604.3	0.0	−647.3
−2.2	−526.3	−1.1	−705.5	−0.8	−1087.4	−1.2	−1113.0	−1.0	−1471.6
−3.0	−544.1	−3.2	−902.2	−1.3	−1129.1	−5.0	−1306.5	−4.8	−1600.0
−5.0	−596.0	−6.1	−1062.8	−5.3	−1293.6	−10.7	−1438.8	−10.0	−1712.5
−8.5	−694.5	−11.6	−1212.4	−15.2	−1516.7	−22.6	−1668.1	−18.0	−1874.2
−12.0	−776.3	−25.6	−1510.9	−27.9	−1760.0	−51.7	−2183.8	−28.9	−2079.9
−25.6	−1011.4	−36.3	−1728.7	−38.3	−1941.5	−78.5	−2651.5	−52.6	−2514.8
−26.1	−1022.7	−45.0	−1884.9	−44.5	−2062.7	−134.1	−3455.4	−78.5	−2968.5
−47.4	−1379.6	−53.9	−2047.1	−54.2	−2234.2	−191.2	−4068.8	−124.8	−3673.2
−78.5	−1882.8	−78.5	−2490.5	−63.8	−2397.5			−193.2	−4441.8
−127.0	−2540.7	−123.6	−3213.8	−78.5	−2645.6				
−184.3	−3128.0	−184.7	−3926.1						

TABLE II

HEAT CAPACITY PER GRAM OF GEL, IN CALORIES

Temp., °C.	Dry gelatin	Gel							
		87.5%	67.7%	61.4%	58.1%	45.3%	24.0%	20.9%	9.0%
25.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.0	−7.12	−8.15	−15.92	−14.77	−16.08	−19.88	−22.09	−22.51	−24.05
−0.6	—	—	—	—	—	—	−40.40	—	—
−0.6	—	—	—	—	—	—	−54.14	—	—
−1.0	—	—	—	—	—	−22.65	−61.54	—	−86.64
−1.2	—	—	—	—	—	—	—	−63.56	—
−2.0	—	—	—	—	—	−27.78	—	−68.22	−88.96
−3.0	—	—	—	−16.16	−18.45	−33.38	−68.54	−71.48	−90.65
−5.0	—	—	—	−20.20	−21.18	−40.42	−71.88	−75.66	−92.73
−10.0	−9.27	−11.29	−21.39	−25.66	−29.16	−46.68	−76.38	−80.18	−95.97
−20.0	—	—	—	−31.36	−34.73	−52.34	−82.50	−85.36	−101.3
−40.0	−15.67	−19.81	−37.00	−39.92	−43.35	−61.49	−91.49	−93.80	−110.0
−60.0	—	—	—	—	—	—	−99.84	—	—
−78.5	—	—	—	—	—	—	−106.3	—	—
−80.0	−24.42	−28.54	−51.53	−55.36	−60.28	−76.50	—	−110.4	−126.3
−120.0	−30.94	−36.03	−61.87	−67.95	−71.74	−89.60	—	−123.0	−139.2
−180.0	−39.17	−42.29	−73.31	−79.24	−81.90	−101.40	—	−135.4	−151.5

for the sake of completeness. At the top of each column is given the concentration of the gel and the weight of the sample used. Values for the heat capacity of the container alone are also included. The values for the 24% gel are the average of at least two determinations each. All the values at 0° C. are also the average of two or more determinations. In other cases, experiments were repeated only when the behavior of the apparatus was erratic and gave reason to doubt the accuracy of the result, or when the transfer of the container from the constant temperature bath to the calorimeter was not successful. These experimental values were all plotted and gave smooth curves as illustrated in Fig. 2. The curves for the 24% and the 67% gels have been omitted from the figure to avoid confusion.

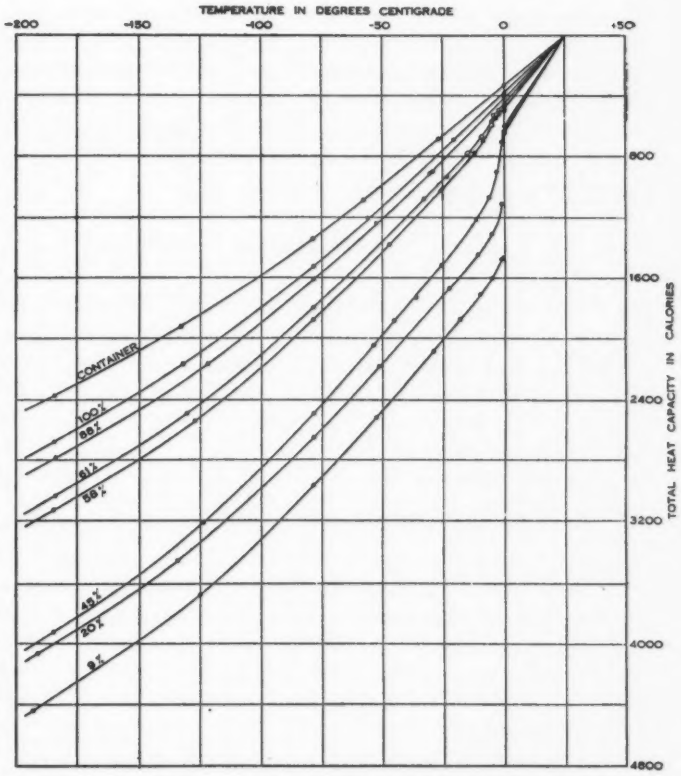

FIG. 2. *Experimental heat capacity curves for container plus gel.*

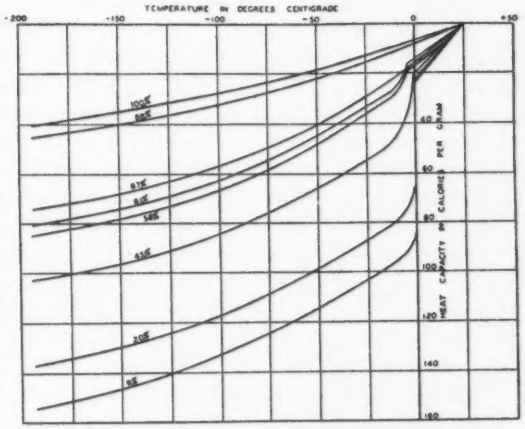

FIG. 3. *Heat capacity curves for gelatin gels.*

From these curves the values for the heat capacity of each of the gels at different temperatures were obtained by subtracting the value for the container at the same temperature. The weight of the sample of gel being known in each case, the heat capacity per gram of gel at different temperatures was calculated. These calculated values are given in Table II and are plotted (omitting the 24% gel) in Fig. 3. All values used in subsequent calculations were read from these smooth curves.

Discussion of Results

Freezing in Gels

From the general appearance of the heat capacity curves in Fig. 3, it is apparent that freezing certainly occurs in gels up to and including 61% gelatin. This is indicated by the definite break in the heat capacity curve which occurs a little below 0° C. The absence of such a break in the curves for the 67% and 87.5% gels apparently means that in these cases there is no freezing at any temperature.

The sharp breaks near 0° C. in the curves for the more dilute gels are evidently due to the latent heat of fusion of the water that is frozen in these gels. In all cases up to 61% gelatin the direction of the curve changes very rapidly between about −1° C. and −25° C. Evidently there is a gradual freezing of water taking place over this range of temperature. The curves are similar to those which would be obtained with a solution of a crystalloid of concentration less than that of the eutectic mixture, over the range between the freezing point and the eutectic temperature (7). However, it cannot be inferred that the mechanism of the freezing point lowering is the same in the two cases.

In the gel, the water is apparently held by forces which vary in intensity, so that the amount of the water which is frozen at a given temperature depends on how much of it can be freed from these forces at that temperature (5). It is well known that when gelatin swells in water there is an enormous contraction in the total volume of gelatin and imbibed water (10). To cause such a contraction in the volume of the water alone would require a tremendous pressure. It is possible therefore, that a portion of the water in a gel is acted upon by forces which are analogous to pressure forces. That water which is under greatest "pressure" would freeze only at the lower temperatures, and the temperature at which any portion of the water could be frozen would be dependent on the pressure acting upon it.

The behavior of the water on freezing these gels is shown more clearly by calculating the heat capacity at various temperatures of the water associated with one gram of dry gelatin. This is done by multiplying the figures read from the smooth curves of Fig. 3 by the weight of gel which contains one gram of dry gelatin, and then subtracting the heat capacity of the dry gelatin. The results are shown in Table III and are represented graphically in Fig. 4 in the form of a family of isothermals, the weight of the water associated with one gram of gelatin being plotted against its heat capacity.

TABLE III

HEAT CAPACITY TO 25° C. OF WATER ASSOCIATED WITH ONE GRAM OF GELATIN

% Gel	Gm. water	0° C.	−10° C.	−30° C.	−50° C.	−100° C.	−180° C.
9.0	10.11	260.7	1058.0	1165.2	1257.0	1449.4	1650.2
20.9	3.79	101.2	374.8	418.8	455.8	532.0	609.4
24.0	3.18	85.9	310.4	351.5	382.5	—	—
45.3	1.21	37.4	94.2	111.9	126.4	155.0	186.7
58.1	0.72	21.5	41.2	54.3	64.7	84.4	103.9
61.4	0.63	17.5	32.9	45.2	54.5	72.5	90.4
67.7	0.48	16.6	22.8	34.0	42.6	56.3	70.1
87.5	0.14	2.9	4.0	5.8	6.9	8.6	10.3

Beyond 0.6 gm. water per gm. dry gelatin, the isothermals are straight lines. The slope of each line gives the heat capacity of the "free" water in the gel between the corresponding temperature and 25° C. The slopes correspond very well with the measured heat capacity per gram of ice (including latent heat of fusion) as shown in Table IV. The figures for the heat capacity of ice were read from the smooth curve obtained by plotting the measurements of Barnes and Maass (1). These go only as low as −78.5° C. and the curve was extended to −180° C. by employing the results of Maass and Waldbauer (6) which agree exactly with those of Barnes and Maass at the higher temperatures.

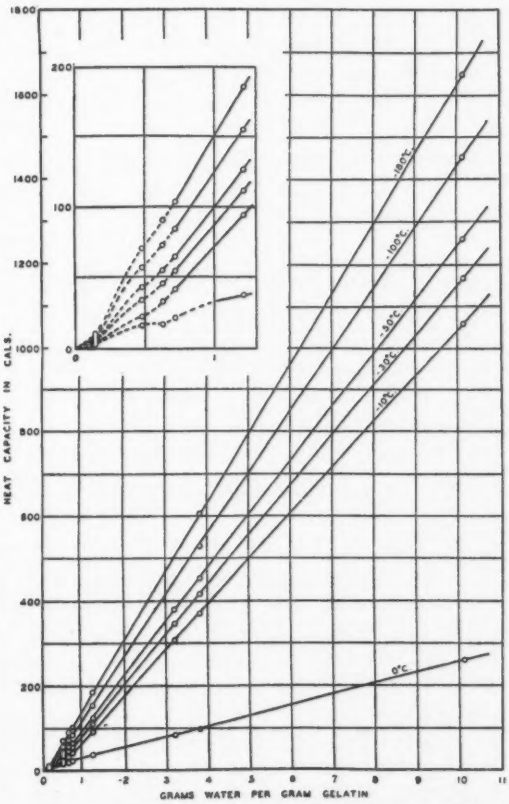

FIG. 4. *Isothermals for heat capacity of water associated with one gram of dry gelatin in gels of various concentrations.*

The point at which the isothermals deviate from the straight line should indicate the amount of water which is regarded as "bound", *i.e.*, which does not display the same heat capacity as "free" water. This is approximately 0.6 gm. per gm. dry gelatin but the data obtained do not admit of defining more closely than this the point where the line begins to bend.

TABLE IV

COMPARISON OF SLOPES OF ISOTHERMALS (FIG. 4) WITH HEAT CAPACITY OF ICE

Temp., °C.	0	−10	−30	−50	−100	−180
Slope of isothermal	25.23	108.3	118.3	127.0	145.3	164.5
Heat capacity of ice, cal.	25.05	109.3	118.6	127.3	146.2	166.3

The results are displayed a little differently in Table V and in Fig. 5, where the heat capacity per gram of the water in a gel is plotted against the weight of gelatin associated with one gram of water. This gives another set of isothermals.

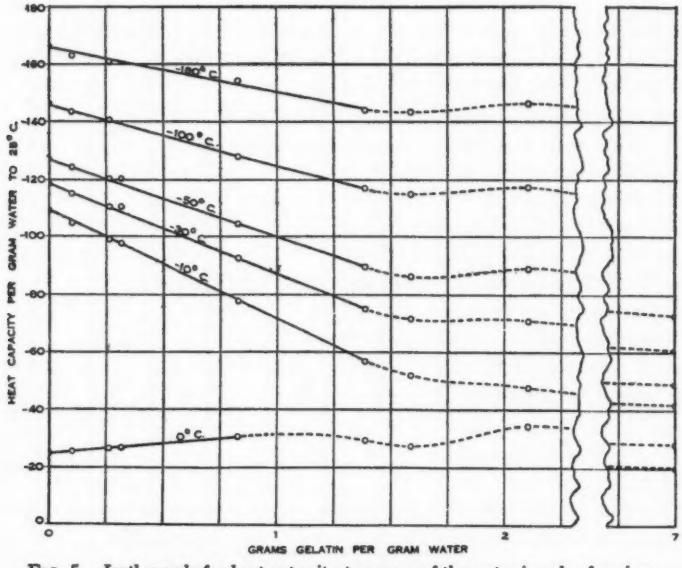

FIG. 5. *Isothermals for heat capacity per gram of the water in gels of various concentrations.*

TABLE V

HEAT CAPACITY PER GRAM TO 25° C. OF WATER IN GELATIN GELS

% Gel	Gm. gelatin per gm. water	0° C.	−10° C.	−30° C.	−50° C.	−100° C.	−180° C.
0.0	0.0	25.05	109.3	118.6	127.3	146.2	166.3
9.0	0.099	25.8	104.6	115.2	124.4	143.4	163.1
20.9	0.264	26.7	99.0	110.5	120.4	140.6	161.1
24.0	0.316	27.0	97.7	110.6	120.4	—	—
45.3	0.828	31.0	77.9	92.6	104.5	128.2	154.4
58.1	1.39	29.9	57.0	75.0	89.8	117.2	144.2
61.4	1.59	27.8	52.0	71.9	86.5	115.2	143.6
67.7	2.11	34.7	47.7	71.1	89.1	117.6	146.4
87.5	7.00	20.1	28.3	41.8	48.8	60.8	72.8

If all the water in the gels were "free" water, its heat capacity would not be affected by the presence of the gelatin and these isothermals would be horizontal straight lines, the height of each above the axis being given by the heat capacity of ice (or water) at the corresponding temperature. The downward slope of the actual isothermals, for temperatures below the freezing point, is due to the decreased heat capacity of the bound portion of the water, which remains unfrozen. They appear to be straight lines up to 1.39 gm. of gelatin per gm. of water (58.1% gel). That is, up to this concentration the decrease in heat capacity caused by adding gelatin to the water is directly proportional to the amount of gelatin added. The inference would seem to be that the amount of bound water is also directly proportional to the amount of gelatin added or, in other words, the amount of water bound per gram of gelatin is independent of the gel concentration up to the limit stated. The same inference may be drawn from the agreement already mentioned between the slopes of the straight lines in Fig. 4 and the heat capacity of ice.

The 0° isothermal of Fig. 5 is discussed below.

Calculation of "Bound" Water

In the previous paper (3) a method of calculating unfrozen or "bound" water was derived. It was based on the following equation,

$$H_{obs.} = aH_g + xH_z + (1-a-x)H_i - \lambda ,$$

where a represents the amount of dry gelatin and x the amount of bound water per gram of gel; $H_{obs.}$ is the measured heat capacity of the gel and H_g, H_z, and H_i the heat capacity per gram of dry gelatin, of bound water and of ice, respectively. The quantity λ was introduced to take into account any evolution of heat which might occur when the frozen portion of the water is reabsorbed into the gel after it has melted. On the basis of Rosenbohm's measurements (12) on the heat of swelling of gelatin, it was assumed that if the unfrozen portion of the water was not less than 0.5 gm. per gm. of dry gelatin (7, 8, 9), λ could be neglected.

H_g, the heat capacity of the dry gelatin, is obtainable from the data in Table II. Values of H_i are given by Barnes and Maass (1) down to −78.5° C. and by Maass and Waldbauer (6) to −180° C. It was thought that an estimate of H_z, the average heat capacity of the bound water, could be obtained from measurements on a gel which showed no break in the heat capacity curve and thus in which, presumably, no freezing occurred. For instance, subtracting the heat capacity of 0.875 gm. of dry gelatin from the heat capacity per gram of the 87.5% gel would give the heat capacity of 0.125 gm. of water bound in this gel. It was realized that, if there are different stages or degrees of binding, the heat capacity of one gram of bound water in an 87.5% gel would not necessarily be the same as the heat capacity of one gram of bound water in a more dilute gel. But it was thought that measurements on a gel containing nearly the maximum amount of non-

freezable water should give a value for the average heat capacity of the water which could be applied to the unfrozen portion of the water in more dilute gels.

This was based on the assumption that that water which is bound in such a way as to remain unfrozen would remain similarly bound at the final temperature of the calorimeter, 25° C. Inspection of the 0° isothermal in Fig. 5 reveals a flaw in this assumption. It is to be expected that the specific heat of the bound portion of the water should be less than 1, since bound water presumably should possess fewer degrees of freedom than free water. Hence the average specific heat of the water contained in a gel, which includes bound water, should be less than 1. In other words, the heat capacity per gram of the water in a gel, even above the freezing point, should be less than that of free water. This inference was verified for the 87.5% gel, but in all the others the heat capacity of the water between 0° and 25° C. proved to be greater than that of free water. This is apparent in Fig. 5 and is also shown by Table VI in which are given the average specific heats obtained by dividing the heat capacities in the third column of Table V by 25.

TABLE VI

AVERAGE SPECIFIC HEAT OF WATER IN GELS BETWEEN 0° AND 25° C.

% Gel	9.0	20.9	24.0	45.3	58.1	61.4	67.7	87.5
Av. sp. ht.	1.03	1.07	1.08	1.24	1.19	1.11	1.38	0.80

The heat capacity of a gel between 0° and 25° C. is greater than the sum of the heat capacities of the water and the gelatin. The increase is proportional to the amount of gelatin per gram of water up to a gel concentration of 45%, since the 0° isothermal of Fig. 5 is a straight line up to that point. Putting it in another way, the increase in heat capacity per gram of gelatin is constant up to a gel concentration of 45%, which is apparent from Fig. 4, where the 0° isothermal is also a straight line within this limit of concentration.

Evidently the process of cooling a gel from 25° to 0° C. results in some change in the gelatin-water relation which is accompanied by the evolution of heat. This might result from an increased binding of water as the temperature is lowered. That this is possible is shown by the work of Neville, Theis and K'Burg (10). They have found that the volume contraction of gelatin gels during the process of swelling increases with lowering of the temperature. Such an increased volume contraction apparently means greater hydration or firmer binding. This process should be accompanied by the evolution of heat since the imbibition of water by gelatin is an exothermic process. Upon warming the gel from 0° to 25° C. the converse would take place, namely the absorption of heat. Hence the measured values for the heat capacities at 0° C. would include this effect, which would explain the unexpectedly high values for the apparent specific heat of the water.

The average specific heat of the water in a 67% gel is found to be greater than 1 down to −80° C. This may be due entirely to the increase in intensity of binding continuing throughout this range of temperature or it may be due to a gradual freezing of part of the water. From the shape of the heat capacity curve (Fig. 3) for this gel, it is impossible to state definitely that there is no freezing. Certainly no break in the curve appears. But there is the possibility of a gradual freezing similar to that which appears to take place in the more dilute gels between the temperature where freezing begins and about −25° C. Some evidence that no freezing occurs was obtained by measuring the heat capacity of this gel at −10° C., first by previous immersion in a bath at −78.5° C., and then by cooling slowly to −10° C. from room temperature. In the latter case supercooling rather than freezing should occur. All the results, however, fell on the same smooth curve.

Another anomalous result of the measurements on the 67% gel is that its heat capacity at 0° C. proves to be greater than that of the 61% gel. This may be seen by reference to Fig. 3 and Table II. The following is advanced as a possible explanation. It has already been suggested that a lowering of the temperature from 25° to 0° C. results in an increased intensity of binding, accompanied by the evolution of heat. The amount of heat evolved during this process will depend on the intensity of the binding forces brought into play (12). In a 67% gel at 25° C. all the water is presumably more or less bound by the gelatin, but as it is cooled to 0° C. an increase in the intensity of binding occurs and heat is evolved. If "binding" is pictured as a surface phenomenon occurring at the boundary of the gelatin particles, it may be assumed that not all the available surface forces are being employed by water molecules at 25° C. As the gel is cooled, additional surface forces are brought into play. Now if it is assumed that, when more water is added at 25° C. to form a more dilute gel, these additional surface forces are brought into action at that temperature, it follows that the increase in intensity of binding on cooling the gel to 0° C., and the consequent evolution of heat, will be less than in the case of the more concentrated gel (compare Ref. (2), page 176). This would be in accord with the experimental observations.

The calculation of bound water from calorimetric measurements is thus complicated by the fact that when the gel is cooled, there occurs, accompanied by evolution of heat, some change in the gelatin-water relation which can hardly be identified with the freezing of water in the gel since it is observed above 0° C. If the same heat effect continues below the freezing point, it is experimentally indistinguishable from the latent heat of freezing. The existence of this effect was quite unsuspected at the beginning of the present research and it has not been recognized at all by previous investigators. Robinson (11), for instance, calculates the specific heat of tissues by means of the formula, $S = h_w f_w + h_s f_s$, where h_w and h_s are the specific heats of water and of the solid portion of the material respectively and f_w and f_s the respective fractions of the total weight. It is evident from Fig. 5 and Table VI that this

equation is not applicable to gelatin gels and this fact renders doubtful its validity in the case of other colloidal systems.

It is clear that the measurements on the 67.7% gel do not provide the hoped-for basis for the estimation of the average heat capacity of the bound water. Values based on the results for the 87.5% gel might be used as was done in the tentative calculations of the previous paper but this procedure involves some rather doubtful assumptions. The complete interpretation of the data here presented calls for further measurements in the region of 50–80% gel concentration and also for detailed and accurate measurements of the heat of swelling at different temperatures. The only figures for the heat of swelling at present available are those of Rosenbohm (12) which were obtained with a Bunsen ice calorimeter and apply only to the temperature 0° C. Further work along these lines is contemplated and it is hoped that the required data may be obtained.

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GRAVITATIONAL AND MAGNETOMETRIC SURVEYS OF THE ONAKAWANA LIGNITE AND GRAND RAPIDS SIDERITE DEPOSITS[1]

By A. H. Miller[2]

Abstract

A traverse about three miles in length was made with a torsion balance over the lignite deposit at Onakawana in northern Ontario, where it has been outlined by extensive drilling over an area of a few square miles. The drilling shows that it is overlain with 50 to 100 ft. of boulder clay and is imbedded in Cretaceous clay of considerably greater density than the coal. The traverse crossed the seam in three places. At each crossing a definite lowering in the value of gravity was observed. The boundary of the deposit was shown along two parts of the traverse by the large and definite effect on the gradient of gravity. Owing to the fact that as a rule the stations were as far as 100 metres apart and that the drill holes were usually 1000 ft. or more from one another, uncertainty exists at some stations regarding the respective gravitational effects of variations of the coal seam, on the one hand, and possible irregularities in the boulder clay, on the other. The results indicate that the torsion balance may be of use in outlining such deposits as the Onakawana lignite. A better estimate of the value of the instrument could be made if it had been possible to make a more definite test of the effect of irregularities in the boulder clay.

Numerous magnetometer readings over and in the vicinity of the coal seam showed so little variation that there appears to be little hope that this instrument would be of use in prospecting for the coal, at least in so far as the immediate boundaries are concerned.

A preliminary survey of the Grand Rapids siderite deposit on the Mattagami river was made with vertical and horizontal magnetometers. The iron occurs in limestone which, except at the river, is buried beneath 50 ft. or more of drift. The anomaly produced by the iron does not apparently exceed 100 γ and is complicated by a larger and more regional anomaly which makes definite interpretation of the results difficult. A more detailed survey might be productive of useful results. Magnetometric traverses were made along the rivers from Grand Rapids to James Bay and from Williams Island in the Abitibi to its mouth. A similar magnetic anomaly to that at Grand Rapids was observed at Williams Island. There is quite a marked magnetic anomaly at Moose Factory.

Introduction

This paper contains an account of geophysical investigations by the Dominion Observatory during the season of 1931 at Onakawana, Ontario, and vicinity, in co-operation with the Ontario Research Foundation and the Ontario Department of Mines. Part I deals with the main investigation which consisted of a torsion balance survey of part of the lignite field at Onakawana, having for its object the determination of the applicability of the instrument to the delineation and possible discovery of such deposits.

While the above survey was in progress, magnetometric traverses of the lignite at Onakawana were also made, and a preliminary magnetometric survey was made at Grand Rapids and along the Mattagami, Moose, and Abitibi rivers. The purpose of this latter work was in the first place, if possible, to outline the boundaries of the siderite deposit at the Grand Rapids of the Mattagami river about 25 miles from Onakawana. Failing this, it

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Contribution from the Dominion Observatory, Ottawa, Canada.

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was hoped that sufficient preliminary work would be completed in the time available to make it possible to form an estimate of the applicability of the magnetic method. A report on this work appears in Part II.

The observations with the instruments (except where the contrary is indicated) were taken by the writer. The work in the field was planned and carried out with the joint co-operation of Mr. R. H. Hawkins, Physicist, and Dr. W. S. Dyer, Geologist, representing the above-mentioned institutions. The torsion balance work was done with the Askania instrument of the Geological Survey, and the magnetic work with three Ad. Schmidt variometers of the Observatory and a fourth belonging to the Geological Survey.

The muskeg, standing at an elevation at Onakawana of 175 ft., is a flat surface, having an average fall in elevation northwards of about three feet per mile. At one time following the retreat of the ice sheet, which left a drift deposit over the coal of from 60 to 100 ft., it formed part of James Bay. The submergence below sea-level for a long period resulted in the smoothing out of the top surface of the boulder clay, and left a layer of marine clay about 10 ft. thick. Since the retreat of the sea there has been deposited on the marine clay in recent geologic times the muskeg, which is, as a rule, about four feet in thickness and consists of decayed vegetation and fallen trees. It is partly wooded. The density of the spongy, mossy material forming the upper surface does not exceed 0.2, and except in very dry weather it is water soaked to the top. It presents a springy surface which is pressed down from six inches to a foot at each step, and there is no rigidity offered by the whole four-foot depth of the muskeg.

Owing to the lightness of the lignite compared with the clay in which it is embedded at Onakawana, the possibility is suggested that the torsion balance might be applied with success to the location of deposits of lignite similar to that outlined by drilling at Onakawana. Other conditions favorable to success with the instrument are presented by the very flat terrain, and by the fact that the thickness of the coal seam approaches a magnitude comparable to the depth of overburden, which varies from about 50 to 100 ft. Factors that might operate against success are possible lack of uniformity in the overburden coupled with the possibility of the coal thinning out so gradually from the main body that only comparatively negligible effects might be produced. It was, of course, to determine how far the favorable conditions were offset by the unfavorable influences that the survey was undertaken.

With the exception of the shallow river valleys the geology of the country for many miles around Onakawana is for the most part concealed beneath a mantle of drift. The area thus offers particular scope for prospecting by geophysical methods.

Figs. 1 and 2 show the conditions under which the coal is found, and give an indication of the gravitational effects that may be expected from the lignite. Fig. 1 is taken from a section supplied by Mr. T. F. Sutherland, Acting Deputy Minister of Mines, Toronto. The densities are averages of several determinations in each case by Mr. R. H. Hawkins. Fig. 1 shows

only the geology near the surface, which is, in all likelihood, responsible for the greater part of the local gravitational effects in the vicinity of the coal. Depending on the inclination and continuity of the strata, comparatively less local effect may be expected from the contact (Fig. 2) of the Cretaceous with the Devonian. More regional effects may be produced by the more deep-seated geology but it is probable that these effects, if they exist, are quite small in the region covered.

Fig. 2 is a reproduction of Fig. 3 of Dyer's paper (1) on the stratigraphy and structural geology of Moose River basin. Information on the geology of the deposit may be obtained from Dyer's papers (1, 2).

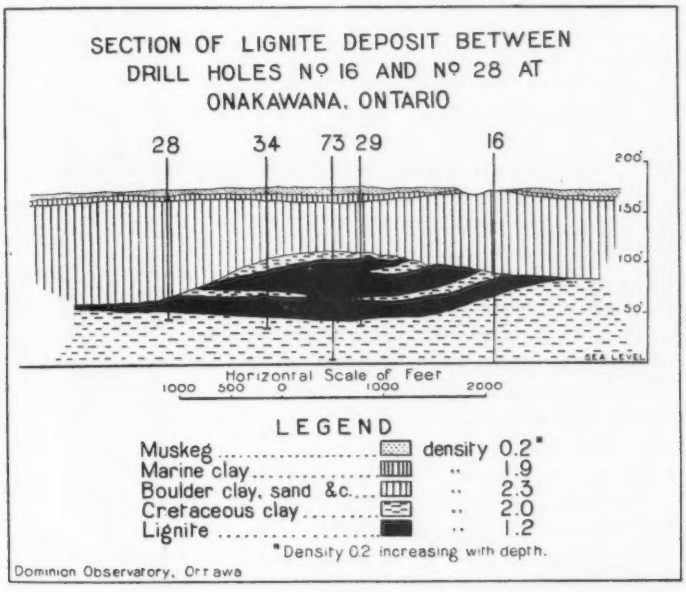


FIG. 1

Part I—Torsion Balance Survey at Onakawana

Setting Up the Instrument

Setting up a delicate instrument for the purpose of observation became the first problem in connection with the work. A satisfactory method was as follows. Three-quarter inch metal rods, about 7 ft. in length, were pushed through to the marine clay and then driven down 18 to 24 in. into it. The tops were set in the form of a triangle and thus formed a tripod with the base plate of the instrument. The tops of the rods were braced with a wooden triangle and a metal wire carrying a turnbuckle. Even on moderately windy days the set-up was slightly disturbed by vibration. A visual instrument was tried but could not be used on account of the vibration caused by the move-

ments of the observer after he had entered the hut. It is possible that with a hut of different construction it might be practicable to use this type of instrument.

Computations of the terrain effect at over 50 stations established the fact that at stations in the open muskeg the effects are so small that they can be safely neglected.

Observations and Discussion of Results

Fig. 3 discloses a number of sharp and rather irregular changes in the observed gravitational effects. This is particularly true of stations in the vicinity of D.H.* 31 and, roughly speaking, of those lying between it and D.H. 19. These results indicate corresponding irregularities in the subterranean densities. Whether these are attributable to variations in the nature of the drift itself, or in the thickness of the coal seam, or a combination of both, is very difficult, if not impossible, to determine from the survey. As we approach D.H. 32 and follow the survey along to the terminus at D.H. 28 there is less irregularity. This is particularly true of the results where the coal seam is thickest, that is, to the north of the Onakawana river in the vicinity of shaft No. 4. Southern boundaries of the coal in apparent agreement with the results of drilling are indicated by the large effects observed in the vicinity of D.H. 26 and 16. With the exception of the gradients observed between D.H. 33 and 29 (these may be caused by an increase in the overburden, a possibility indicated by D.H. 35), the gradients over the area south of the Onakawana are small, and that at the station to the south of D.H. 28 is in agreement with the thickening in the boulder clay and thinning in the coal seam revealed by the drill hole. Generally, the magnitude and direction of the H.D.T. over the area in which the thick seam is found, agree with that to be expected from the information supplied by the drilling.

*Drill hole.

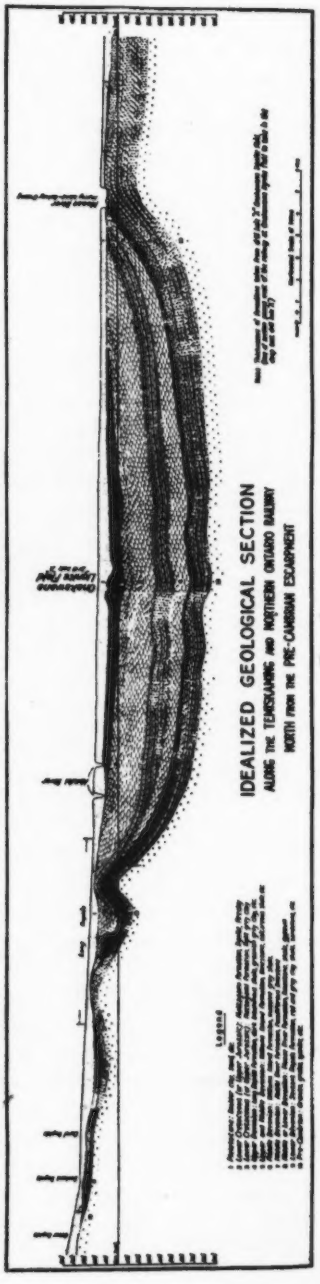

IDEALIZED GEOLOGICAL SECTION
ALONG THE TEMISKAMING AND NORTHERN ONTARIO RAILWAY
NORTH FROM THE PRE-CAMBRIAN ESCARPMENT

FIG. 2

With one exception, the gradients and H.D.T's between the station due east of shaft No. 1 and D.H. 20 are small and maintain on the average uniform directions. If there is coal in the vicinity of the traverse, the indication is that it lies to the east. In agreement with the drilling a barren area is indicated in the vicinity of D.H. 30. It is probable that the gravitational effects in

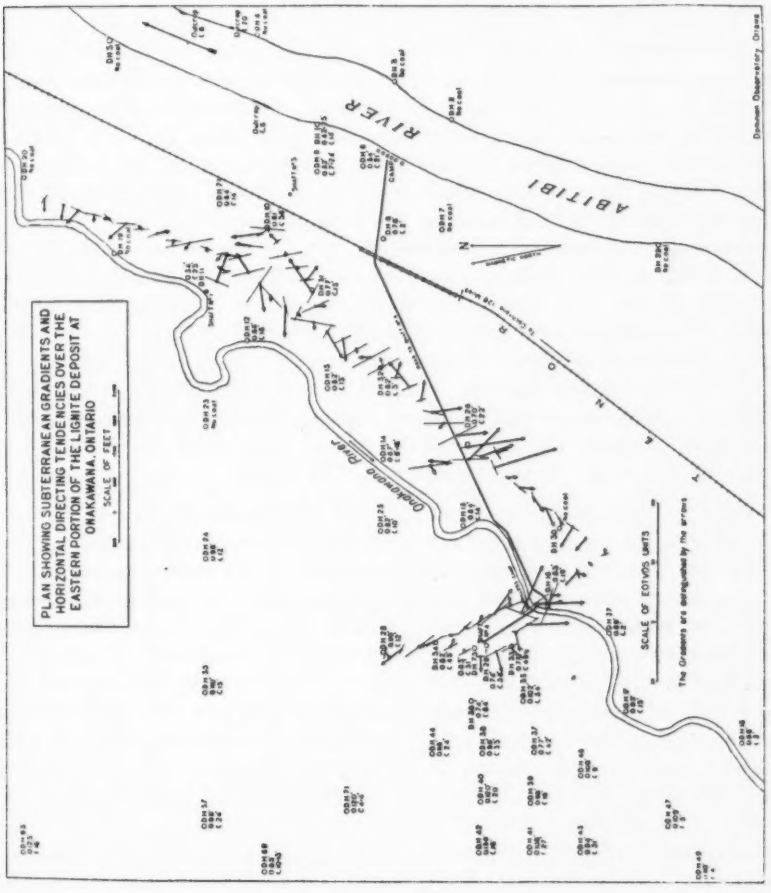

PLAN SHOWING SUBTERRANEAN GRADIENTS AND HORIZONTAL DIRECTING TENDENCIES OVER THE EASTERN PORTION OF THE LIGNITE DEPOSIT AT OMAKAWANA, ONTARIO
SCALE OF FEET
RIVER
ABITIBI
Onakawana Point
SCALE OF EÖTVÖS UNITS
The Gradients are designated by the arrows

FIG. 3

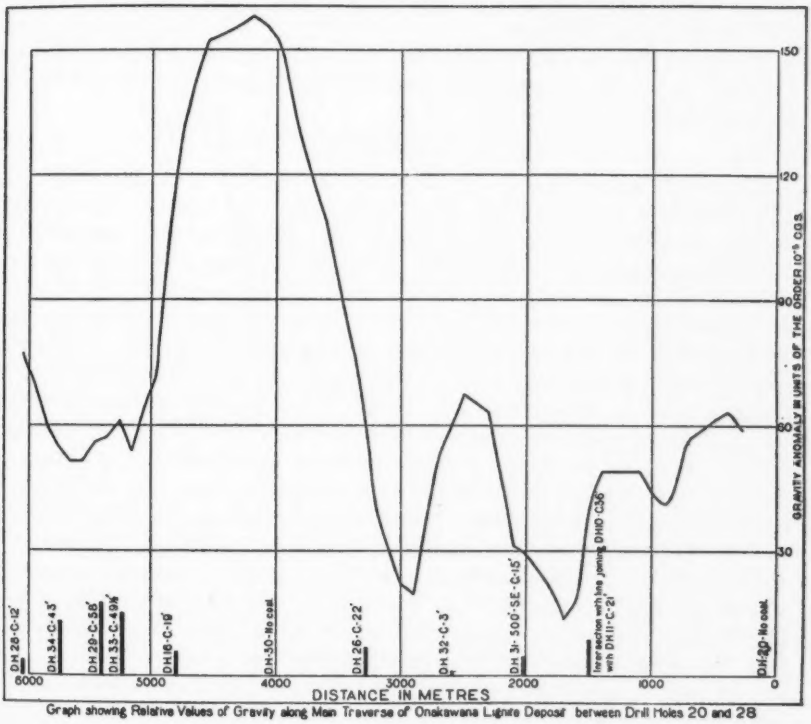
this area are due mainly to irregularities in the boulder clay, and it is likely that the traverse of this region gives a better indication of the nature and magnitude of these particular effects than does any other part of the survey. Along the main traverse in the vicinity of D.H. 31 there are two stations in which there are sudden reversals in the curvature amounting to about 40 Eötvös units. Without the establishment of other stations near them, it is

difficult to say to what they are due. They might be due to local thickening of the seam of about 20 ft. or to the overburden changing from clay to either sand or gravel. If a channel with vertical edges (length great compared with its width) were imagined near the top surface of the overburden, then it would be necessary for the channel to be just so deep that the side of it subtended an angle of about 20° at the stations in order for it to produce the gravitational effect mentioned in the curvature. This could be accounted for in many ways. For example, a channel in the boulder clay filled with sand or gravel of thickness 14 ft. immediately below the marine clay with edges at horizontal distances of 14 ft. on either side of the station would be sufficient to produce the effect. Furthermore, the effect would be practically negligible at external points more than 85 ft. from either edge. A thickening of the coal seam is likely to take place at the expense of the boulder clay, thus bringing the coal seam nearer the surface. Owing to this fact and to the fact also that faces of a seam in the form of a rectangular block produce opposite gravitational effects at points outside the block, a narrow seam of coal will produce quite a local effect too. A local thickening of 15 ft. over a width of 120 ft. in the main coal seam would produce the required effect at a station situated over the centre, and an effect of not more than 10 Eötvös units at external points 60 ft. from either edge, and of course still less than this for points farther distant. D.H. 31 is evidently situated on the easterly edge of a disturbance and so placed that the maximum effect is produced upon the gradient. This may be due to the same cause that results in the large curvature in the second station to the north.

Cross Traverse at Station 35 or D.H. 26 and Stations in their Vicinity. In considering the results for this short traverse and those in the vicinity of D.H. 16, it may be remarked that the magnitudes of the gradients and H.D.T's are such that they might be caused by the amount of overburden and coal indicated at the drill holes, which, at D.H. 26, is 70 and 22 ft. respectively. For an abrupt boundary at D.H. 26 there would theoretically be a maximum gradient of about 44 Eötvös units, which is very close to the observed value of about 50. However, the rate of change of gradient and the H.D.T. are too gradual to be in accord with the conditions specified above. To satisfy all the observed requirements of an abrupt boundary, it would be necessary that the mean depth of the material causing the disturbance should lie at a depth of about 100 metres. For example, a block of coal 31 metres thick with an overburden of 86 metres would be required to account for the effects. The existence of such a block is, of course, not likely. If we suppose that D.H. 26 is on a sloping edge of coal, as it appears to be from the observations, and that in the vicinity to the north of the drill hole the seam thickens, it can be shown that theoretically the observations could be approximately satisfied by a seam with a sloping edge of about 1 in 10, and a thickness of about 50 ft. The effects at the two most northerly stations, owing to their distance, can be only slightly affected in so far as the gradient is concerned by the assumed conditions in the vicinity of D.H. 26. The results at these stations

represent new conditions that are encountered in their vicinity. Qualitatively at least an increase extending for a considerable lateral distance in the mass of the boulder clay before reaching the last station and a decrease at the last station would produce the observed effects.

The Gravity Anomaly Curve. Fig. 4 presents the integrated effects of the gradients along the main traverse, obtained by multiplying the average value of the gradients resolved along the traverse by the distance between the stations; the values represent the differences in gravity between the



Graph showing Relative Values of Gravity along Main Traverse of Onakawana Lignite Deposit between Drill Holes 20 and 28

FIG. 4

stations reduced to a common elevation, and corrected for terrain, topography, and latitude. A curve drawn through the tops of the columns (Fig. 4) representing the thickness of the coal, would form a more or less inverted image of the gravity anomaly curve. The coal regions are regions of low gravity and the barren areas of high gravity. In the region of thickest (most) coal, to the left in the figure, notwithstanding the fact that there is a large relative (compared with the immediate vicinity) fall in gravity, it is not the lowest value when compared with other parts of the traverse between D.H. 26 and 20. Most likely this is due to accumulated error along the traverse

(particularly in parts where the gravity effects are large) in computing the value of gravity. The closing error of the loop of stations through D.H. 10 and 31 was found to be large, *viz.*, 80×10^{-5} c.g.s. units. The closing of the loop at shaft No. 4 was more satisfactory (less than 20×10^{-5} c.g.s. units). The closing errors of the stations situated on the two loops have in each case been distributed evenly around the loops, and the adopted values have been adjusted to satisfy this requirement. In the loop passing through D.H. 10 the stations are too far apart to give reliable differences in gravity, particularly if instead of being arranged in the form of a closed loop they had been laid out in a linear traverse.

It is interesting to note that the fall of gravity between D.H. 30 and D.H. 29 (shaft No. 4) is just sufficient to account for an extensive seam of coal 60 ft. in thickness.

Conclusion

While sufficient work has not been done to afford a complete solution of the problem, a great deal has been learned. In searching for new deposits and in the detailed outlining of known deposits, the torsion balance may have some application. Indication of favorable areas which, in the final analysis, might or might not contain coal, might reasonably be expected. In exploratory work it would be advisable to place the stations closer together. In a traverse, two lines with the stations at staggered intervals would probably be best. It was found when working with one instrument that 10 to 12 stations could be established in a week. Working with two instruments a party of four could easily run in a week's time a traverse of one kilometre with stations at 50 metre intervals. Before undertaking any extensive exploration it would be desirable to do some further work in a similar drift-covered region where it is certain there is no coal or where the thickness of the coal is well established from numerous drill holes. Although the nature of the topography simplifies the work by eliminating the necessity for terraining, this is offset by the fact that it is difficult to get about in the muskeg, at least in that part of the season best suited for the work. In view of this and the fact that observations with the torsion balance are a slow, tedious process, and keeping in mind the nature of the results obtained so far and that the instrument is as a rule better suited for investigation in detail, it is doubtful whether any extensive exploration with the instrument is to be recommended, except in regions where there is a fair probability of finding coal, and then only in close co-operation with a geologist in order that useless efforts may be avoided and due consideration given to the geology of the region in so far as it is known.

Part II—Magnetometric Surveys

Magnetometric Survey of the Onakawana Lignite Deposit

Traverses of the coal seam with a vertical magnetometer were made by Mr. R. H. Hawkins. Little variation in the intensity was noted. The small anomalies that were observed did not appear to be related to the existence of the lignite.

Grand Rapids Siderite Deposit

The results of the observations at Grand Rapids are shown in Figs. 5, 6, and 7. In Fig. 5 the contact of the ore with the limestone is along the river bank at the point marked *C*, and extends along the bank for a few feet to the east of pit No. 1. It outcrops again between *E* and *F*. The ore forms

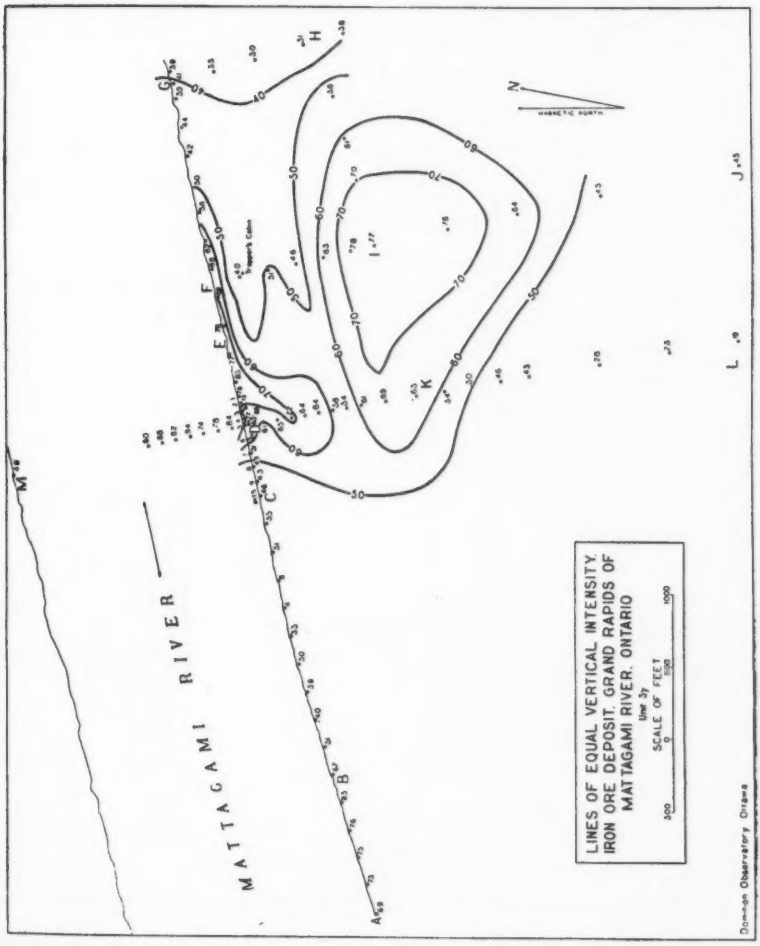


FIG. 5

part of the river bed. The observations in the river were taken on ore in the shallower parts at low water. Immediately to the south of the river bank, the ore disappears under a heavy covering of drift. Between *A* and *B* the bank is formed of ferruginous sands and several iron concretions lying loose along the bank were noted by Dr. Dyer.

The ore is only slightly magnetic and varies in magnetic susceptibility. Samples measured by an approximate method gave values ranging between nearly zero and 350 $\times$ 10^{-6} c.g.s. units. Assuming the ore to be uniformly magnetized, the maximum vertical effect over the body would not likely

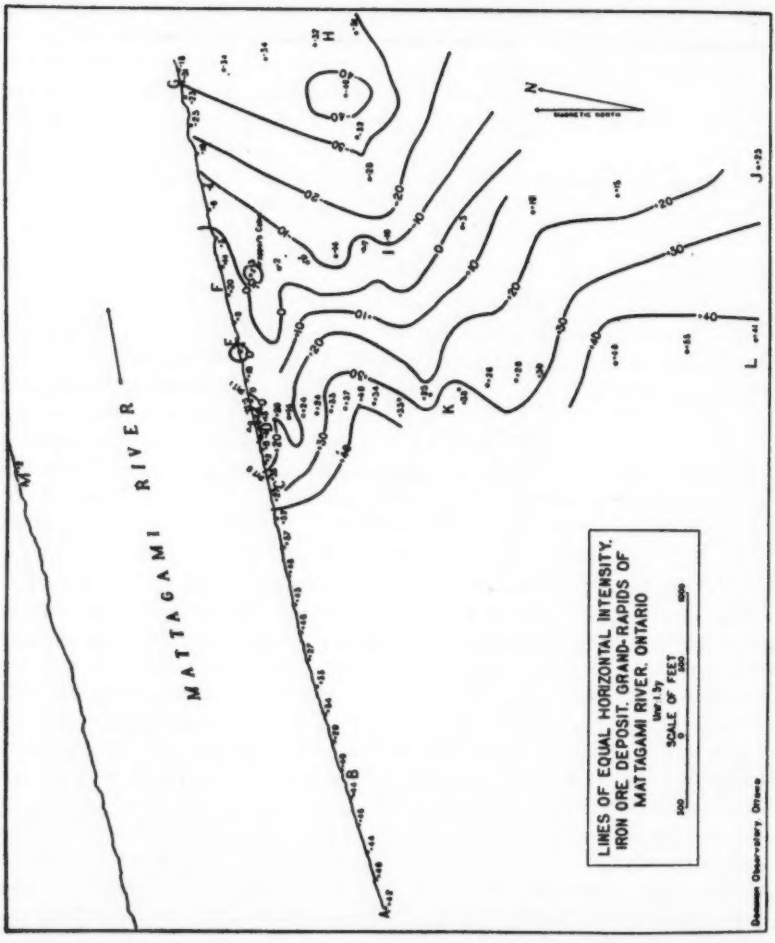


FIG. 6

exceed 140γ, taking the susceptibility to be not greater than the above value and with the point of observation far enough from the ore boundaries to escape effects due to magnetic charges on the sides of the ore body. The observed maximum positive anomaly produced by the ore does not apparently exceed 100γ.

Values given in Figs. 5 and 6 are expressed in terms of one-tenth division of the corrected readings of the respective instruments. For Fig. 7 the

normal values were determined by averaging the values obtained at 500-ft. intervals along the main traverses indicated by the lettering. The adopted normal values are 55 for the vertical intensity and 13 for the horizontal. Fig. 5 discloses three regions of high vertical intensity, one between *A* and *B*, another occupying the greater part of the area covered by the outcrop of ore, and a third centering around *I*. Fig. 7 indicates that the cause of the

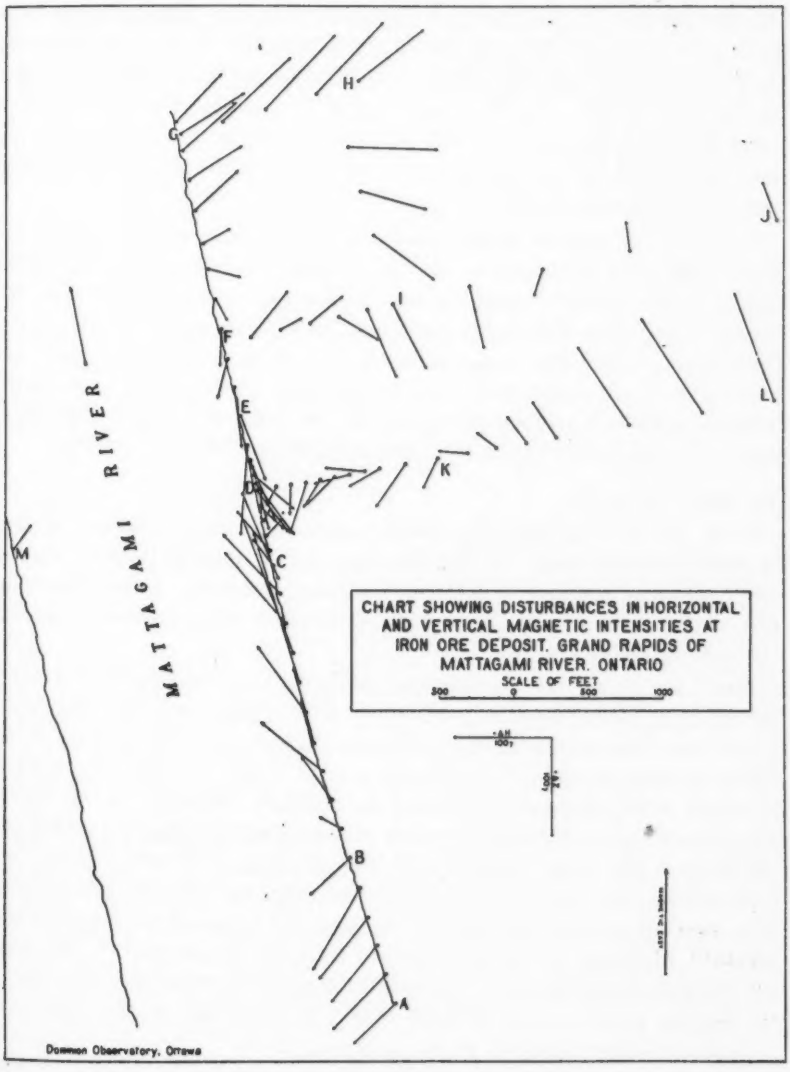


FIG. 7

disturbance between *A* and *B* is located in the direction of the river and that the cause of the disturbance at *I* is more deep-seated than that of the disturbance south of *D*. The centre of disturbance at *D* is indicated at a depth of about 200 ft. which includes up to 100 ft. of drift. It is not likely that the magnetic effect of the iron produces all the anomalies indicated on the charts. The effect of the iron is apparently small and for the most part is masked by a larger and more regional effect. However, it may be that the effect to the south of *D* is due to the iron, and that the extension of the first 55 contour line immediately to the south of the river indicates approximately the southern boundary of the ore. On the other hand, it is clear that the ore exerts little magnetic effect, perhaps too little to render its detection certain. A more detailed, methodical and careful survey laid down in the form of a grid might lead to more certain results.

Records at 15 stations where observations were repeated from two to nine times, show that the probable error of a single observation with the vertical magnetometer was 0.5 scale division, equivalent to an error of 15γ. The corresponding error with the horizontal magnetometer was 0.6 scale division, equivalent to 9γ. The larger probable error with the vertical instrument may be due to the fact that the base instrument available for the vertical measurements was less sensitive than the corresponding horizontal instrument, the scale values in the two cases being 60 and 15γ, respectively.

The River Traverses

A traverse with stations 500 to 1000 ft. apart was run to a distance of about 2½ miles southerly from *J* in the direction of *I J* to investigate the regional effect that was apparent in the results already obtained. It was found that with minor fluctuations the horizontal intensity kept increasing and the vertical decreasing (Fig. 8).

Along the rivers are encountered geological conditions similar to those prevailing at Grand Rapids (particularly at Williams Island on the Abitibi) where there is no reason to suspect the presence of ore. The results, not corrected by base instrument, are shown in Figs. 9 and 10. The readings were corrected from observed variations in intensity observed at Agincourt.* The normal or zero value for the river traverses was obtained by taking from the curve in Fig. 9 the observed values at intervals of 2½ miles. Values were corrected for the latitude variations in the magnetic intensities, as obtained by a least square solution from the values of the observed intensities at New Liskeard, Mattawa, Ft. Albany, Moose Factory, and Cochrane.** In making the solution no account was taken of the longitude variation. The result of the solution gave 9.1γ per mile for *H* and 6.8γ per mile for *Z*. The results

*Supplied by the Director of the Meteorological Service.

**The values for these places were supplied by Mr. C. A. French of the Dominion Observatory staff.

reveal a decided local anomaly at Moose Factory at about one-half to three-quarters of a mile south along the water's edge from the Hudson Bay post. The ore exposure at Grand Rapids is on an anticline whose axis runs almost due east and west. It is exposed again on crossing the Abitibi River at Williams Island, about 11 miles east. Figs. 9 and 10 show a similar magnetic effect at the two crossings.

The positions of the points of observation along the river were obtained by projection along the astronomical meridian. It must be borne in mind in interpreting the results that the profiles are not the ideal ones along the magnetic meridian. The horizontal anomaly and the resultant disturbance

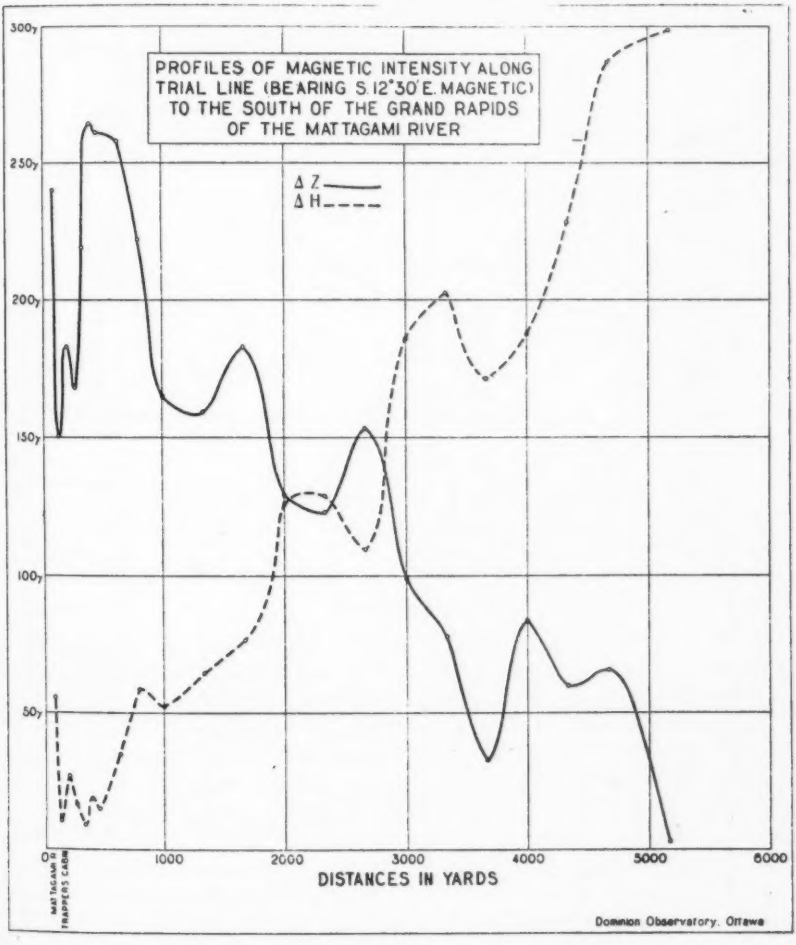


FIG. 8

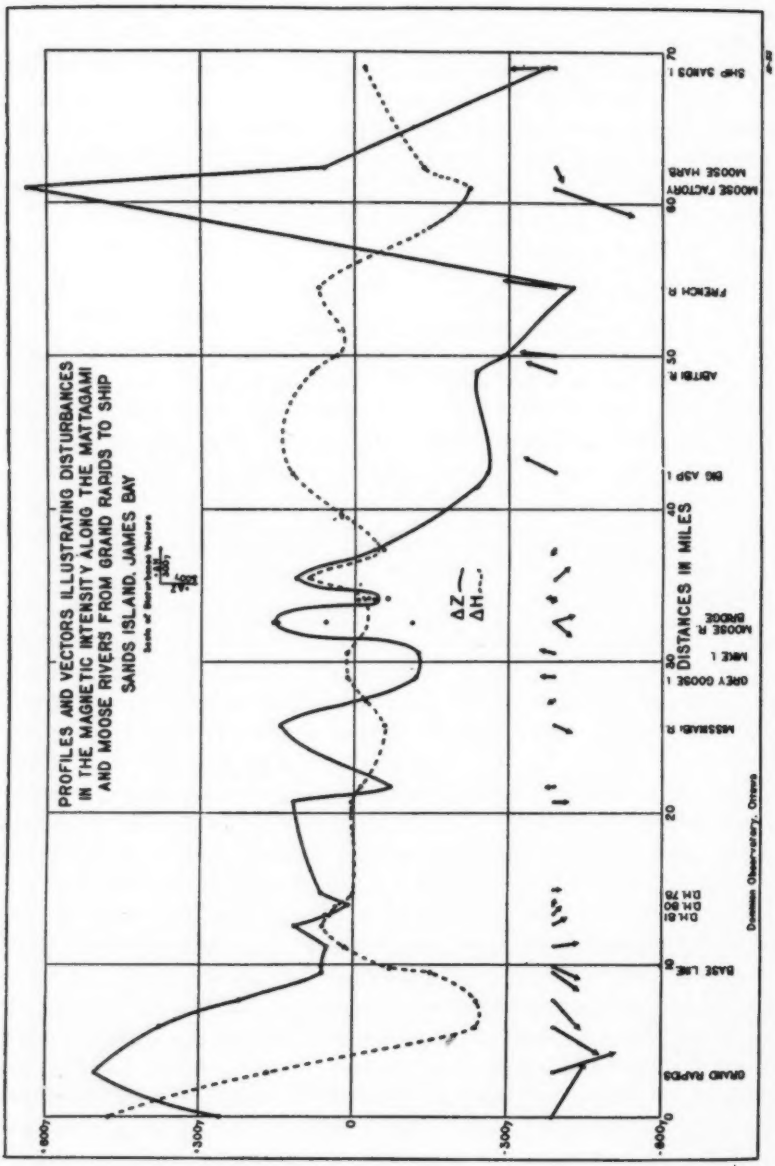

PROFILES AND VECTORS ILLUSTRATING DISTURBANCES IN THE MAGNETIC INTENSITY ALONG THE MATTAGAMI AND MOOSE RIVERS FROM GRAND RAPIDS TO SHIP SANDS ISLAND, JAMES BAY
Scale of Disturbance Vectors
ΔZ
ΔH
+600γ
+300γ
0
-300γ
-600γ
DISTANCES IN MILES
GRAND RAPIDS
BASE LINE
DM 81
DM 80
MISSINABI R.
GREY GOOSE I.
MARE I.
MOOSE R.
BIG ASP I.
ABITIBI R.
FRENCH R.
MOOSE FACTORY
MOOSE HARB.
SHIP SANDS I.
Dominion Observatory, Ottawa

FIG. 9

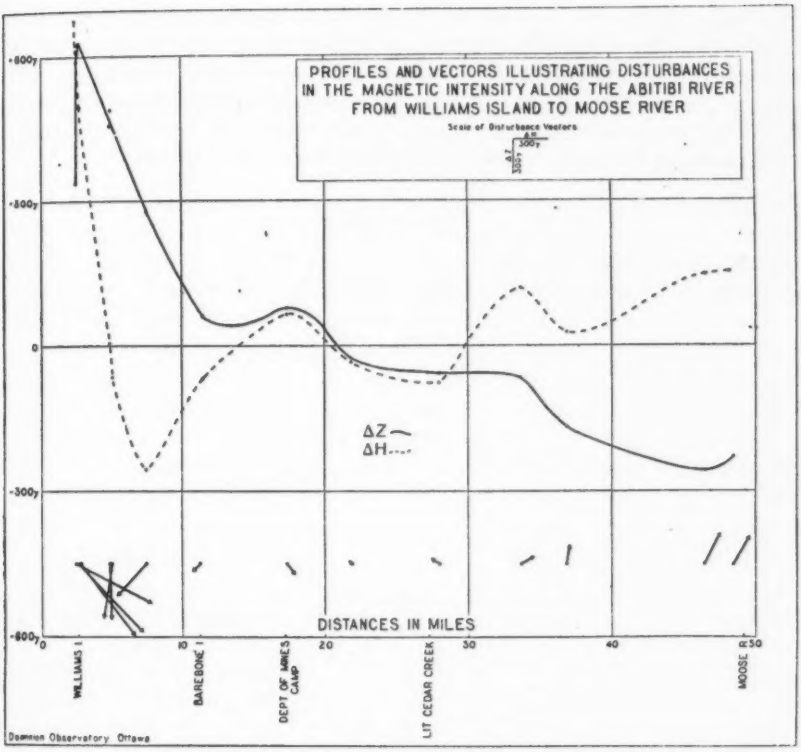


FIG. 10

are in each case disturbances in the plane of the magnetic meridian, and not in the plane of the traverse. This also applies to the iron ore deposit at Grand Rapids (Fig. 7).

CONCLUSION

The results of the magnetic observations at Grand Rapids give some, though indefinite, indication of a southern boundary of the ore a few hundred feet from the river bank. The magnetic anomaly produced by the ore body is small, and is complicated by that due to a more deep-seated cause. If further magnetic work is contemplated, it would be best to plan the observations to cover the ground in the form of a grid, with one of the directions in the magnetic meridian. Accurate determinations of the temperature coefficients and of the scale values of the instruments from time to time would be essential, and it might be found advisable to repeat, preferably on different days, all or most of the observations. The diurnal variation might be evaluated either by an independent observer with another set of instruments at the base, or by returning at frequent intervals to a selected base point with the

field instruments. Even in the former case observations with the field instrument should be taken at the beginning and end of each day at some selected point in order to follow the changes that take place in the instruments themselves. To survey a square mile with observations at 200-ft. intervals, obtaining both magnetic components and possibly also the declinations, would require about 30 days; at 100-ft. intervals, about four months.

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REVIEWS AND NOTES

A CONVENIENT TUBE ELECTROMETER[1]

By V. G. Smith[2]

Recently the School of Hygiene of this university desired to make routine pH measurements using a glass electrode, and it was preferable to make use of the Cambridge potentiometer and Leeds and Northrup 2420–D galvanometer already available. As this will probably be the situation in many cases the circuit used may be of considerable interest.

Convenience in use was kept in mind in designing the circuit. No attempt was made to compensate for battery e.m.f. changes (3, 4) or variations in filament emission (1). It was found that in 15 min. or less the drift of the galvanometer was so slow that it did not interfere with the measurements. Of course this requires that the battery be well charged and then discharged to a condition of steady voltage. The same thing applies to the potentiometer battery.

The circuit is shown in Fig. 1, which also shows the method of connecting to the potentiometer. The tube is a General Electric F.P.–54, supplied entirely from a 12-volt battery. A milliammeter (100 ma.) with a red line at 90 ma. permits the accurate adjustment of the filament current and hence of all voltages.

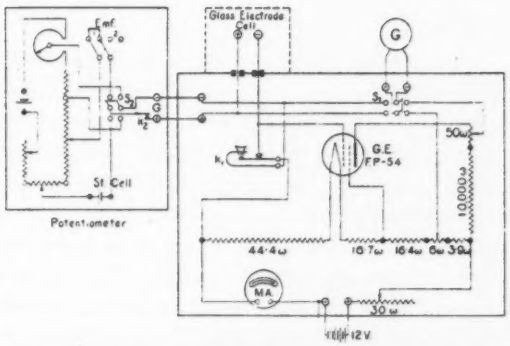

FIG. 1. *The electrometer tube circuit, showing the method of connecting to the potentiometer so that the potentiometer may be easily calibrated and used for other purposes without disconnecting the amplifier.*

By means of the switch S_1 the galvanometer may be connected either in the plate circuit of the tube or directly to the galvanometer terminals of the potentiometer. For neatness the switch S_1 is a D.P.D.T. telephone jack switch. To calibrate the potentiometer it is necessary only to close S_2 to the calibration position (down) and S_1 to the potentiometer (left). Furthermore, with S_1 to the potentiometer (left) and using e.m.f. terminals No. 2, the potentiometer and galvanometer may be used for other work without disconnecting any wires.

[1] *Manuscript received January 24, 1934.*
Contribution from the Department of Electrical Engineering, University of Toronto, Toronto, Ontario, Canada.

[2] *Assistant Professor of Electrical Engineering, University of Toronto.*

When the instrument is used as an amplifier the galvanometer is connected to the plate circuit by the switch S_1 and balanced to zero by means of the two potentiometer rheostats. The 6-ohm rheostat is the coarse adjustment and the 50-ohm rheostat the fine. By short circuiting e.m.f. No. 1 a known potential difference may be obtained across the galvanometer terminals of the potentiometer. With k_2 closed, the pressing of k_1 inserts the potential difference of the potentiometer and the e.m.f. of the glass electrode cell into the control grid circuit in series. When these two are equal and opposite no change in the plate current takes place and hence there is no change in the galvanometer deflection. With a 1000-ohm galvanometer about 90% of the change in the plate current passes through the galvanometer. The potentiometer therefore indicates directly the e.m.f. of the cell when, with k_2 closed, pressing k_1 does not *change* the galvanometer reading. It does not matter whether the galvanometer actually reads zero or not, only the change is important.

It is convenient to lock k_2 closed. If it is of a non-locking type a lead weight may be used. When this is done S_2 should be moved before S_1 when calibrating the potentiometer, for if S_1 is moved first the galvanometer is connected directly across the potentiometer and a violent, time wasting swing results.

It is essential that the grid circuit shall not be opened when inserting the potentiometer and cell, otherwise a violent deflection takes place as the grid proceeds to take on its free-grid potential (2). This means that k_1 must close the new path before opening the old, which results in a temporary short circuit of the potentiometer and glass electrode cell. When finally balanced this is immaterial, but to minimize the possibility of polarization in the preliminary adjustments it is desirable to have the duration of the short circuit as short as possible. For this purpose the "close-before-open" type of switch is used at k_1, permitting extremely short durations of short circuit and yet assuring the proper sequence of events. Such a switch is shown in principle in the diagram.

The whole circuit is shielded in a copper box and for convenience everything is mounted from the lid. It is necessary that the key k_1 shall be a shielded key. In this instrument it is made of brass and fits snugly into a brass sleeve which is grounded to the box. A bakelite insert for appearance and a bakelite tip for insulation are added.

The stationary contact of the key k_1 is amber insulated and both insulators where the cell wires leave the box are made of amber though only the negative wire had to be so insulated. Wires connected to the control grid are kept clear of all other insulation. The box is reasonably air-tight and provision is made for drying the air with phosphorus pentoxide. Two nipples are provided so that air may be circulated if desired.

A convenient method of testing for insulation leaks is to use a standard cell with a 100 megohm grid-leak in series with it in place of the glass electrode cell. As the correct e.m.f. is known any errors caused by leakage may be detected.

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